EXHIBIT 50

IW 7696335

THE UNITED STATES OF AMERICA

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October 11, 2018

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APPLICATION NUMBER: ***13/967,163***
FILING DATE: ***August 14, 2013***
PATENT NUMBER: ***8,629,111***
ISSUE DATE: ***January 14, 2014***

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

P. SWAIN
Certifying Officer

Docket No. 17618CON6B (AP)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Acheampong, *et al.*	Examiner: TBA
Serial No.: TBA	Group Art Unit: TBA
Filed: Herewith	Confirmation No. TBA
For: METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	Customer No.: 51957

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prior to examining the above-referenced application, please amend the specification as described on page 2 of this paper, and please amend the claims as described on pages 3-6 of this paper. Remarks follow on page 7.

Amendments to the Specification

Please replace page 1, lines 5-10 of the specification filed herewith with the following amended paragraph:

This application is a continuation of copending U.S. Application Serial No. 13/961,828 filed August 7, 2013, which is a continuation of copending U.S. Application Serial No. 11/897,177, filed August 28, 2007, which is a continuation of U.S. Application Serial No. 10/927,857, filed August 27, 2004, now abandoned, which claimed the benefit of U.S. Provisional Application No. 60/503,137 filed September 15, 2003, which ~~is~~ are incorporated in ~~its~~ their entirety herein by reference.

Please replace page 4, line 25 – page 5, line 3 of the specification filed herewith with the following amended paragraph:

The present methods are useful in treating any suitable condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphy1actic endophthalmitis, uveitis, vernal conjunctivitis, atopic keratoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome. Cyclosporin has been found as effective in treating immune mediated keratoconjunctivitis sicca (KCS or dry eye disease) in a patient suffering therefrom. The activity of cyclosporins is as an immunosuppressant and in the enhancement or restoring of lacrimal gland tearing. Other conditions that can be treated with cyclosporin components include an absolute or partial deficiency in aqueous tear production (keratoconjunctivitis sicca, or KCS). Topical administration to a patient's tear deficient eye can increase tear production in the eye. The treatment can further serve to correct corneal and conjunctival disorders exacerbated by tear deficiency and KCS, such as corneal scarring, corneal ulceration, inflammation of the cornea or conjunctiva, filamentary keratisis, mucopurulent discharge and vascularization of the cornea.

Amendments to the claims

The following list of claims will replace all previous versions of claims presented in this application:

1. – 36. (Canceled)

37. (New) A topical ophthalmic emulsion for treating an eye of a human having KCS, wherein the topical ophthalmic emulsion comprises cyclosporin A in an amount of about 0.05% by weight, polysorbate 80, Pemulen, water, and castor oil in an amount of about 1.25% by weight; and

wherein the topical ophthalmic emulsion is therapeutically effective in treating KCS.

38. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion further comprises a tonicity agent or a demulcent component.

39. (New) The topical ophthalmic emulsion of Claim 38, wherein the tonicity agent or the demulcent component is glycerine.

40. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion further comprises a buffer.

41. (New) The topical ophthalmic emulsion of Claim 40, wherein the buffer is sodium hydroxide.

42. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion further comprises glycerine and a buffer.

43. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion comprises polysorbate 80 in an amount of about 1.0% by weight.

44. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion comprises Pemulen in an amount of about 0.05% by weight.

45. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion further comprises glycerine in an amount of about 2.2% by weight, water, and a buffer.

46. (New) The topical ophthalmic emulsion of Claim 45, wherein the buffer is sodium hydroxide.

47. (New) The topical ophthalmic emulsion of Claim 37, wherein, when the topical ophthalmic emulsion is administered to an eye of a human in an effective amount in treating KCS, the blood of the human has substantially no detectable concentration of cyclosporin A.

48. (New) The topical ophthalmic emulsion of Claim 42, wherein the topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

49. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion is as substantially therapeutically effective as an emulsion comprising cyclosporin A in an amount of 0.1% by weight and castor oil in an amount of 1.25% by weight.

50. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion achieves at least as much therapeutic effectiveness as an emulsion comprising cyclosporin A in an amount of 0.1% by weight and castor oil in an amount of 1.25% by weight.

51. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion breaks down more quickly in the eye of a human, once administered to the eye

of the human, thereby reducing vision distortion in the eye of the human as compared to an emulsion that contains only 50% as much castor oil.

52. (New) The topical ophthalmic emulsion of Claim 37, wherein the topical ophthalmic emulsion, when administered to the eye of a human, demonstrates a reduction in adverse events in the human, relative to an emulsion comprising cyclosporin A in an amount of 0.1% by weight and castor oil in an amount of 1.25% by weight.

53. (New) The topical ophthalmic emulsion of Claim 52, wherein the adverse events include side effects.

54. (New) A topical ophthalmic emulsion for treating an eye of a human, wherein the topical ophthalmic emulsion increases tear production in the eye of a human, and wherein the topical ophthalmic emulsion comprises:

cyclosporin A in an amount of about 0.05% by weight;

castor oil in an amount of about 1.25% by weight;

polysorbate 80 in an amount of about 1.0% by weight;

Pemulen in an amount of about 0.05% by weight;

a tonicity component or a demulcent component in an amount of about 2.2% by weight;

a buffer; and

water.

55. (New) The topical ophthalmic emulsion of Claim 54, wherein the buffer is sodium hydroxide.

56. (New) The topical ophthalmic emulsion of Claim 54, wherein the tonicity component or the demulcent component is glycerine.

57. (New) The topical ophthalmic emulsion of Claim 54, wherein, when the topical ophthalmic emulsion is administered to an eye of a human in an effective amount to increase tear production, the blood of the human has substantially no detectable concentration of the cyclosporin A.

58. (New) The topical ophthalmic emulsion of Claim 54, wherein the topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

59. (New) The topical ophthalmic emulsion of Claim 54, wherein the topical ophthalmic emulsion is effective in treating KCS.

60. (New) A topical ophthalmic emulsion for treating an eye of a human, the topical ophthalmic emulsion comprising:

cyclosporin A in an amount of about 0.05% by weight;
castor oil in an amount of about 1.25% by weight;
polysorbate 80 in an amount of about 1.0% by weight;
Pemulen in an amount of about 0.05% by weight;
glycerine in an amount of about 2.2% by weight;
sodium hydroxide; and
water;
wherein the emulsion is effective in treating KCS.

61. (New) The topical ophthalmic emulsion of Claim 60, wherein the topical ophthalmic emulsion has a pH in the range of about 7.2 to about 7.6.

REMARKS

The applicants have canceled claims 1-36 and have added claims 37-61. Support for the limitations recited in the new claims may be found throughout the specification, and at least at page 4, line 25 – page 5, line 14, page 26, lines 5-19, and page 27, lines 4-31 of the application specification filed herewith.

Support for the amendment to the specification at page 4, line 25 – page 5, line 3 may be found, at least, in U.S. Patent Nos. 5,474,979 and 6,254,860, which were previously incorporated by reference in the present application specification at page 1, lines 18-21. The amendment contains no new matter.

The claims of the present application may vary in scope from the claims pursued in the parent applications. To the extent any prior amendments or characterizations of the scope of any claim, or the specification, or referenced art could be construed as a disclaimer of any subject matter supported by the present disclosure, the Applicants hereby rescind and retract such disclaimer.

Specifically, the Applicants would like to bring to the Examiner's attention comments made in the Response filed on June 15, 2009 in U.S. Patent Application Serial No. 10/927,857 (now abandoned) and comments made in the Amendment filed on June 15, 2009 in U.S. Patent Application Serial No. 11/897,177 (currently pending) regarding U.S. Patent No. 5,474,979 and the present application specification. Since these comments have been filed, the Applicants have collected evidence that supports the patentability of the pending claims.

The Commissioner is hereby authorized to charge any fees required or necessary for the filing, processing or entering of this paper or any of the enclosed papers, and to refund any overpayment, to deposit account 01-0885.

Respectfully submitted,

/Laura L. Wine/

Date: August 14, 2013

Laura L. Wine
Attorney of Record
Registration Number 68,681

Please direct all inquiries and correspondence to:
Laura L. Wine, Esq.
Allergan, Inc.
2525 Dupont Drive, T2-7H
Irvine, California 92612
Tel: (714) 246-6996 Fax: (714) 246-4249

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 13/961,828

filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Andrew Acheampong Date (Optional) : ____________

Signature: [signature]

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 13/961,828

filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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LEGAL NAME OF INVENTOR

Inventor: DIANE TANG-LIU Date (Optional) : ____________

Signature: Diane Tang-Liu

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS Docket No.: 17618CON6(AP)

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 13/961,828

filed on 8/7/2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

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LEGAL NAME OF INVENTOR

Inventor: DAVID F. POWER Date (Optional) : 8-12-2013

Signature: David F. Power

Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.

Doc code: Oath
Document Description: Oath or declaration filed

PTO/AIA/02 (06-12)
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SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)

Title of Invention	Methods of Providing Therapeutic Effects Using Cyclosporin Components Docket No.: 17618CON6(AP)

This statement is directed to:

☐ The attached application,

OR

☒ United States application or PCT international application number 13/961,828 filed on 8-7-13.

LEGAL NAME of inventor to whom this substitute statement applies:

(*E.g.*, Given Name (first and middle (if any)) and Family Name or Surname)

James N. Chang

Residence (except for a deceased or legally incapacitated inventor):

City	State	Country
Newport Beach	CA	US

Mailing Address (except for a deceased or legally incapacitated inventor):

36 Cervantes

City	State	Zip	Country
Newport Beach	CA	92660	US

I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.

The above-identified application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Relationship to the inventor to whom this substitute statement applies:

☐ Legal Representative (for deceased or legally incapacitated inventor only),
☒ Assignee,
☐ Person to whom the inventor is under an obligation to assign,
☐ Person who otherwise shows a sufficient proprietary interest in the matter (petition under 37 CFR 1.46 is required), or
☐ Joint Inventor.

PTO/SB/AIA02 (06-12)
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SUBSTITUTE STATEMENT

Circumstances permitting execution of this substitute statement:

- [ ] Inventor is deceased,
- [ ] Inventor is under legal incapacity,
- [ ] Inventor cannot be found or reached after diligent effort, or
- [x] Inventor has refused to execute the oath or declaration under 37 CFR 1.63.

If there are joint inventors, please check the appropriate box below:

- [x] An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.

OR

- [ ] An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: Debra D. Condino TITLE: ASSISTANT SECRETARY COMPANY: ALLERGAN, INC. (ASSIGNEE) Date (Optional):

Signature: DCondino

Residence (unless provided in an application data sheet, PTO/AIA/14 or equivalent):

City	State	Country
Irvine	CA	US

Mailing Address (unless provided in an application data sheet, PTO/AIA/14 or equivalent)

2525 Dupont Drive-T2-7H

City	State	Zip	Country
Irvine	CA	92612	US

Note: Use an additional PTO/AIA/02 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in the attached transmittal letter.

[X] I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent): 51957

OR

[ ] I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A or equivalent):

Name	Registration Number	Name	Registration Number

Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:

[X] The address associated with the above-mentioned Customer Number.

OR

[ ] The address associated with Customer Number:

OR

[ ] Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant:

[ ] Inventor or Joint Inventor

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (*e.g.*, a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent			
Signature	D Condino	Date	09/20/2012
Name	Debra D. Condino, Reg. No. 31,007	Telephone	714-246-2388
Title and Company	Assistant Secretary, Allergan, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms for more than one signature, see below *.

[ ] *Total of ____________ forms are submitted.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (03-13)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Andrew Acheampong	Nonprovisional Application Number (if known):	
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, examination fee, and any required excess claims and application size fees are filed with the request or have been already been paid.
2. The application contains or is amended to contain no more than four independent claims and no more than thirty total claims, and no multiple dependent claims.
3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. The executed inventor's oath or declaration is filed with the application. (37 CFR 1.63 and 1.64)

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Laura L. Wine/	Date August 14, 2013
Name (Print/Typed) Laura L. Wine	Practitioner Registration Number 68681

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☑ *Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Filer:	Laura Lee Wine
Attorney Docket Number:	17618CON6B (AP)

Filed as Large Entity

Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Claims:				
Claims in Excess of 20	1202	5	80	400
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Publ. Fee- Early, Voluntary, or Normal	1504	1	300	300
OTHER PUBLICATION PROCESSING FEE	1808	1	130	130

Petition:
Patent-Appeals-and-Interference:
Post-Allowance-and-Post-Issuance:
Extension-of-Time:
Miscellaneous:

Total in USD ($)	**6430**

Electronic Acknowledgement Receipt

EFS ID:	16592584
Application Number:	13967163
International Application Number:	
Confirmation Number:	4274
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Customer Number:	51957
Filer:	Laura Lee Wine
Filer Authorized By:	
Attorney Docket Number:	17618CON6B (AP)
Receipt Date:	14-AUG-2013
Filing Date:	
Time Stamp:	18:33:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$6430
RAM confirmation Number	5973
Deposit Account	010885
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	17618CON6B_ADS.pdf	1505467 45df93d7cb088ac75701b7c82b88a6a4a4c574b4	no	8

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
2		17618BCON6_SPEC.pdf	4359979 e47cc7584c4695688bd25cc9d63b38422505afe2	yes	34

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	28
Claims	29	33
Abstract	34	34

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
3	Miscellaneous Incoming Letter	17618CON6B_POA.pdf	1931210 035a077f1c36b8af6d1b1390dbfdde368b7f1913	no	2

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
4	Power of Attorney	New_POA.pdf	1822911 054720bccc55afbbefb1f91959b4661f3a266bec	no	1

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
5		17618CON6B_Preliminary_Amendment.pdf	107973 48046abd3c5d119bec51babcaf6fa5c14993defc	yes	8

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Specification	2	2

	Claims		3		6
	Applicant Arguments/Remarks Made in an Amendment		7		8
Warnings:					
Information:					
6	Oath or Declaration filed	Dec17618CON6.pdf	5927597 b6fca939a23c997d10c48a656971bfbc1eff1ce0	no	6
Warnings:					
Information:					
7	TrackOne Request	PrioritizedExamination-17618B-CON6.pdf	153242 22511b6906631625dd18d953c97a4817fd6092b4	no	2
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	41933 2d1a23ce6ad442d7249f3212700ce124c5fa138a	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	15850312	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove
Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Andrew		Acheampong	

Residence Information (Select One) ◉ US Residency ◯ Non US Residency ◯ Active US Military Service

City	State/Province	Country of Residence
Irvine	CA	US

Mailing Address of Inventor:

Address 1	16 Wintergreen
Address 2	
City	Irvine
State/Province	CA
Postal Code	92604
Country	US

Inventor 2 Remove
Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Diane	D.	Tang-Liu	

Residence Information (Select One) ◉ US Residency ◯ Non US Residency ◯ Active US Military Service

City	State/Province	Country of Residence
Las Vegas	NV	US

Mailing Address of Inventor:

Address 1	3726 Las Vegas Blvd S. Unit 3303 W
Address 2	
City	Las Vegas
State/Province	NV
Postal Code	89158
Country	US

Inventor 3 Remove
Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	James	N.	Chang	

Residence Information (Select One) ◉ US Residency ◯ Non US Residency ◯ Active US Military Service

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

City	Newport Beach	State/Province	CA	Country of Residence i	US

Mailing Address of Inventor:

Address 1	36 Cervantes		
Address 2			
City	Newport Beach	State/Province	CA
Postal Code	92660	Country i	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	David	F.	Power	

Residence Information (Select One) (●) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Hubert	State/Province	NC	Country of Residence i	US

Mailing Address of Inventor:

Address 1	202 Fox Way N		
Address 2			
City	Hubert	State/Province	NC
Postal Code	28539	Country i	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	51957	
Email Address	patents_ip@allergan.com	Add Email / Remove Email

Application Information:

Title of the Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS		
Attorney Docket Number	17618CON6B (AP)	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	51597		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	13961828	2013-08-07
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13961828	Continuation of	11897177	2007-08-28
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
11897177	Continuation of	10927857	2004-08-27
Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
10927857	non provisional of	60503137	2003-09-15

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	
Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) i the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country i	Filing Date (YYYY-MM-DD)	Access Code i (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

PTO-000027

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date of filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section. Clear

- (●) Assignee
- ( ) Legal Representative under 35 U.S.C. 117
- ( ) Joint Inventor
- ( ) Person to whom the inventor is obligated to assign.
- ( ) Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. [X]

Organization Name	Allergan, Inc.		
Mailing Address Information:			
Address 1	2525 Dupont Drive		
Address 2			
City	Irvine	**State/Province**	CA
Country i	US	Postal Code	92612
Phone Number		Fax Number	

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Email Address	patent_ip@allergan.com

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section only if non-applicant assignee information is desired to be included on the patent application publication in accordance with 37 CFR 1.215(b). Do not include in this section an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest), as the patent application publication will include the name of the applicant(s).

Remove

If the Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications

Signature	/Laura L. Wine/			Date (YYYY-MM-DD)	2013-08-14
First Name	Laura	Last Name	Wine	Registration Number	68681

Additional Signature may be generated within this form by selecting the Add button. Add

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	17618CON6B (AP)
	Application Number	

Title of Invention	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

D-3111CON

METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Related Application

This application is a continuation of U.S. Application Serial No. 10/927,857, filed August 27, 2004, which claimed the benefit of U.S. Provisional Application No. 60/503,137 filed September 15, 2003, which is incorporated in its entirety herein by reference.

Background of the Invention

The present invention relates to methods of providing desired therapeutic effects to humans or animals using compositions including cyclosporin components. More particularly, the invention relates to methods including administering to an eye of a human or animal a therapeutically effective amount of a cyclosporin component to provide a desired therapeutic effect, preferably a desired ophthalmic or ocular therapeutic effect.

The use of cyclosporin-A and cyclosporin A derivatives to treat ophthalmic conditions has been the subject of various patents, for example Ding et al U.S. Patent 5,474,979; Garst U.S. Patent 6,254,860; and Garst U.S. 6,350,442, this disclosure of each of which is incorporated in its entirely herein by reference. In addition, cyclosporin A compositions used in treating ophthalmic conditions is the subject of a number of publications. Such publications include, for example, "Blood concentrations of cyclosporin a during long-term treatment with cyclosporin a ophthalmic emulsions in patients with moderate to severe dry eye disease," Small et al, *J Ocul Pharmacol Ther, 2002 Oct, 18(5):411-8*; "Distribution of

cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs," Acheampong et al, *Curr Eye Res, 1999 Feb, 18(2):91-103b;* "Cyclosporine distribution into the conjunctiva, cornea, lacrimal gland, and systemic blood following topical dosing of cyclosporine to rabbit, dog, and human eyes," Acheampong et al, *Adv Exp Med Biol, 1998, 438:1001-4;* "Preclinical safety studies of cyclosporine ophthalmic emulsion," Angelov et al, *Adv Exp Med Biol, 1998, 438:991-5;* "Cyclosporin & Emulsion & Eye," Stevenson et al, *Ophthalmology, 2000 May, 107(5):967-74;* and "Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group," Sall et al, *Ophthalmology, 2000 Apr, 107(4):631-9.* Each of these publications is incorporated in its entirety herein by reference. In addition, cyclosporin A-containing oil-in-water emulsions have been clinically tested, under conditions of confidentiality, since the mid 1990's in order to obtain U.S. Food and Drug Administration (FDA) regulatory approval.

Examples of useful cyclosporin A-containing emulsions are set out in Ding et al U.S. Patent 5,474,979. Example 1 of this patent shows a series of emulsions in which the ratio of cyclosporin A to castor oil in each of these compositions was 0.08 or greater, except for Composition B, which included 0.2% by weight cyclosporin A and 5% by weight castor oil. The Ding et al patent placed no significance in Composition B relative to Compositions A, C and D of Example 1.

Over time, it has become apparent that cyclosporin A emulsions for ophthalmic use preferably have less than 0.2%

by weight of cyclosporin A. With cyclosporin A concentrations less than 0.2%, the amount of castor oil employed has been reduced since one of the functions of the castor oil is to solubilize the cyclosporin A. Thus, if reduced amounts of cyclosporin are employed, reduced amounts of castor oil are needed to provide effective solubilization of cyclosporin A.

There continues to be a need for providing enhanced methods of treating ophthalmic or ocular conditions with cyclosporin-containing emulsions.

Summary of the Invention

New methods of treating a human or animal using cyclosporin component-containing emulsions have been discovered. Such methods provide substantial overall efficacy in providing desired therapeutic effects. In addition, other important benefits are obtained employing the present methods. For example, patient safety is enhanced. In particular, the present methods provide for reduced risks of side effects and/or drug interactions. Prescribing physicians advantageously have increased flexibility in prescribing such methods and the compositions useful in such methods, for example, because of the reduced risks of harmful side effects and/or drug interactions. The present methods can be easily practiced. In short, the present methods provide substantial and acceptable overall efficacy, together with other advantages, such as increased safety and/or flexibility.

In one aspect of the present invention, the present methods comprise administering to an eye of a human or animal a composition in the form of an emulsion comprising water, a hydrophobic component and a cyclosporin component

in a therapeutically effective amount of less than 0.1% by weight of the composition. The weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

It has been found that the relatively increased amounts of hydrophobic component together with relatively reduced, yet therapeutically effective, amounts of cyclosporin component provide substantial and advantageous benefits. For example, the overall efficacy of the present compositions, for example in treating dry eye disease, is substantially equal to an identical composition in which the cyclosporin component is present in an amount of 0.1% by weight. Further, a relatively high concentration of hydrophobic component is believed to provide for a more quick or rapid breaking down or resolving of the emulsion in the eye, which reduces vision distortion which may be caused by the presence of the emulsion in the eye and/or facilitates the therapeutic effectiveness of the composition. Additionally, and importantly, using reduced amounts of the active cyclosporin component mitigates against undesirable side effects and/or potential drug interactions.

In short, the present invention provides at least one advantageous benefit, and preferably a plurality of advantageous benefits.

The present methods are useful in treating any suitable condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome,

phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome.

Employing reduced concentrations of cyclosporin component, as in the present invention, is advantageously effective to provide the blood of the human or animal under treatment with reduced concentrations of cyclosporin component, preferably with substantially no detectable concentration of the cyclosporin component. The cyclosporin component concentration of blood can be advantageously measured using a validated liquid chromatography/mass spectrometry-mass spectrometry (VLC/MS-MS) analytical method, such as described elsewhere herein.

In one embodiment, in the present methods the blood of the human or animal has concentrations of clyclosporin component of 0.1 ng/ml or less.

Any suitable cyclosporin component effective in the present methods may be used.

Cyclosporins are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. Cyclosporin A, along with several other minor metabolites, cyclosporin B through I, have been identified. In addition, a number of synthetic analogs have been prepared.

In general, commercially available cyclosporins may contain a mixture of several individual cyclosporins which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different substituents or configurations of some of the amino acids.

The term "cyclosporin component" as used herein is intended to include any individual member of the

cyclosporin group and derivatives thereof, as well as mixtures of two or more individual cyclosporins and derivatives thereof.

Particularly preferred cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof. Cyclosporin A is an especially useful cyclosporin component.

Any suitable hydrophobic component may be employed in the present invention. Advantageously, the cyclosporin component is solubilized in the hydrophobic component. The hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions.

The hydrophobic component preferably is present in the emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount of up to about 1.0% by weight or about 1.5% by weight or more of the composition.

Preferably, the hydrophobic component comprises one or more oily materials. Examples of useful oil materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils and the like and mixtures thereof. In a very useful embodiment, the hydrophobic component comprises one or more higher fatty acid glycerides. Excellent results are obtained when the hydrophobic component comprises castor oil.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the compositions. Examples of such other components include, without limitation, emulsifier components, tonicity components,

polyelectrolyte components, surfactant components, viscosity inducing components, acids and/or bases to adjust the pH of the composition, buffer components, preservative components and the like. Components may be employed which are effective to perform two or more functions in the presently useful compositions. For example, components which are effective as both emulsifiers and surfactants may be employed, and/or components which are effective as both polyelectrolyte components and viscosity inducing components may be employed. The specific composition chosen for use in the present invention advantageously is selected taking into account various factors present in the specific application at hand, for example, the desired therapeutic effect to be achieved, the desired properties of the compositions to be employed, the sensitivities of the human or animal to whom the composition is to be administered, and the like factors.

The presently useful compositions advantageously are ophthalmically acceptable. A composition, component or material is ophthalmically acceptable when it is compatible with ocular tissue, that is, it does not cause significant or undue detrimental effects when brought into contact with ocular tissues.

Such compositions have pH's within the physiological range of about 6 to about 10, preferably in a range of about 7.0 to about 8.0 and more preferably in a range of about 7.2 to about 7.6.

The present methods preferably provide for an administering step comprising topically administering the presently useful compositions to the eye or eyes of a human or animal.

Each and every feature described herein, and each and

every combination of two or more of such features, is included within the scope of the present invention provided that the features included in such a combination are not mutually inconsistent.

These and other aspects and advantages of the present invention are apparent in the following detailed description, example and claims.

Detailed Description

The present methods are effective for treating an eye of a human or animal. Such methods, in general, comprise administering, preferably topically administering, to an eye of a human or animal a cyclosporin component-containing emulsion. The emulsion contains water, for example U.S. pure water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the emulsion. In addition, beneficial results have been found when the weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

As noted above, the present administering step preferably includes topically administering the emulsion to the eye of a patient of a human or animal. Such administering may involve a single use of the presently useful compositions, or repeated or periodic use of such compositions, for example, as required or desired to achieve the therapeutic effect to be obtained. The topical administration of the presently useful composition may involve providing the composition in the form of eye drops or similar form or other form so as to facilitate such topical administration.

The present methods have been found to be very

effective in providing the desired therapeutic effect or effects while, at the same time, substantially reducing, or even substantially eliminating, side effects which may result from the presence of the cyclosporin component in the blood of the human or animal being treated, and eye irritation which, in the past, has been caused by the presence of certain components in prior art cyclosporin-containing emulsions. Also, the use of the present compositions which include reduced amounts of the cyclosporin components allow for more frequent administration of the present compositions to achieve the desired therapeutic effect or effects without substantially increasing the risk of side effects and/or eye irritation.

The present methods are useful in treating any condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome.

The frequency of administration and the amount of the presently useful composition to use during each administration varies depending upon the therapeutic effect to be obtained, the severity of the condition being treated and the like factors. The presently useful compositions are designed to allow the prescribing physician substantial flexibility in treating various ocular conditions to achieve the desired therapeutic effect or effects with

reduced risk of side effects and/or eye irritation. Such administration may occur on an as needed basis, for example, in treating or managing dry eye syndrome, on a one time basis or on a repeated or periodic basis once, twice, thrice or more times daily depending on the needs of the human or animal being treated and other factors involved in the application at hand.

One of the important advantages of the present invention is the reduced concentration of the cyclosporin component in the blood of the human or animal as a result of administering the present composition as described herein. One very useful embodiment of the present administering step provides no substantial detectable concentration of cyclosporin component in the blood of the human or animal. Cyclosporin component concentration in blood preferably is determined using a liquid chromatography-mass spectroscopy-mass spectroscopy (LC-MS/MS), which test has a cyclosporin component detection limit of 0.1 ng/ml. Cyclosporin component concentrations below or less than 0.1 ng/ml are therefore considered substantially undetectable.

The LC-MS/MS test is advantageously run as follows.

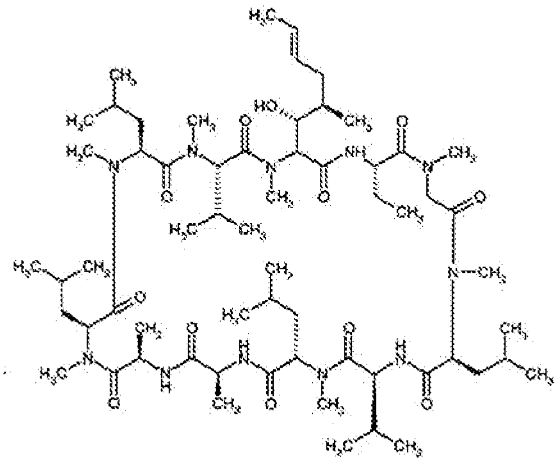
One ml of blood is acidified with 0.2 ml of 0.1 N HCl solution, then extracted with 5 ml of methyl t-butyl ether. After separation from the acidified aqueous layer, the organic phase is neutralized with 2 ml of 0.1 N NaOH, evaporated, reconstituted in a water/acetonitrile-based mobil phase, and injected onto a 2.1 x 50 mm, 3μm pore size C-8 reverse phase high pressure liquid chromatography (HPLC) column (Keystone Scientific, Bellefonte, PA). Compounds are gradient-eluted at 0.2 mL/min and detected using an API III triple quadrupole mass spectrometer with a

turbo-ionspray source (PE-Sciex, Concord, Ontario, Canada). Molecular reaction monitoring enhances the sensitivity and selectivity of this assay. Protonated molecules for the analyte and an internal standard are collisionally dissociated and product ions at m/z 425 are monitored for the analyte and the internal standard. Under these conditions, cyclosporin A and the internal standard cyclosporin G elute with retention times of about 3.8 minutes. The lower limit of quantitation is 0.1 ng/mL, at which concentration the coefficient of variation and deviation from nominal concentration is <15%.

As noted previously, any suitable cyclosporin component effective in the present methods may be employed. Very useful cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof.

The chemical structure for cyclosporin A is represented by Formula 1

Formula I

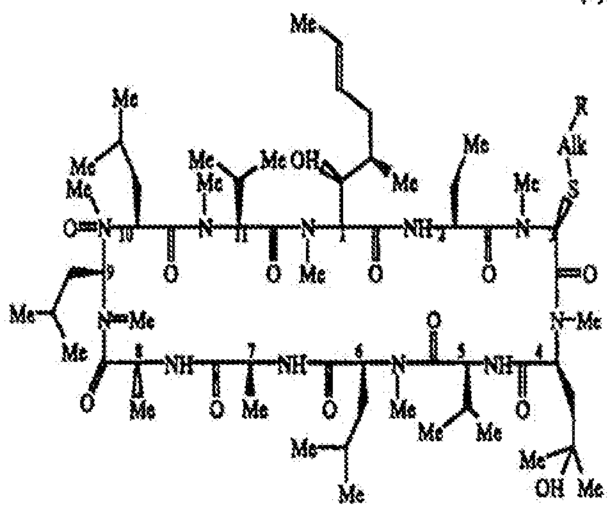


As used herein the term "derivatives" of a cyclosporin refer to compounds having structures sufficiently similar to the cyclosporin so as to function in a manner substantially similar to or substantially identical to the cyclosporin, for example, cyclosporin A, in the present methods. Included, without limitation, within the useful cyclosporin A derivatives are those selected from ((R)-methylthio-Sar)^3-(4'-hydroxy-MeLeu) cyclosporin A, ((R)-(Cyclo)alkylthio-Sar)^3-(4'-hydroxy-MeLeu)^4-cyclosporin A, and ((R)-(Cyclo)alkylthio-Sar)^3-cyclosporin A derivatives described below.

These cyclosporin derivatives are represented by the following general formulas (II), (III), and (IV) respectively:

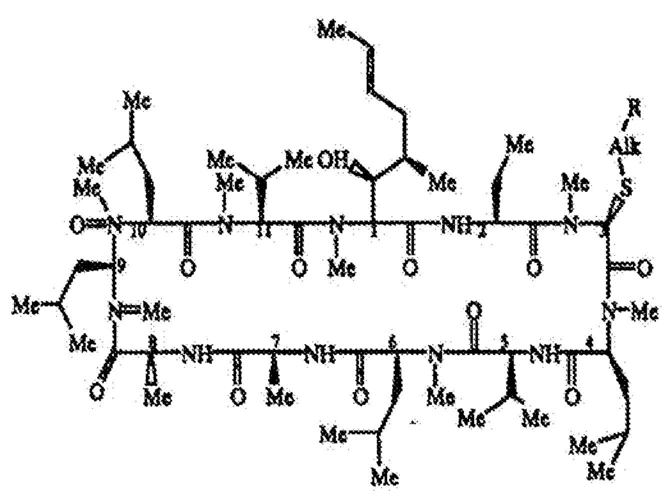
Formula II

(II)



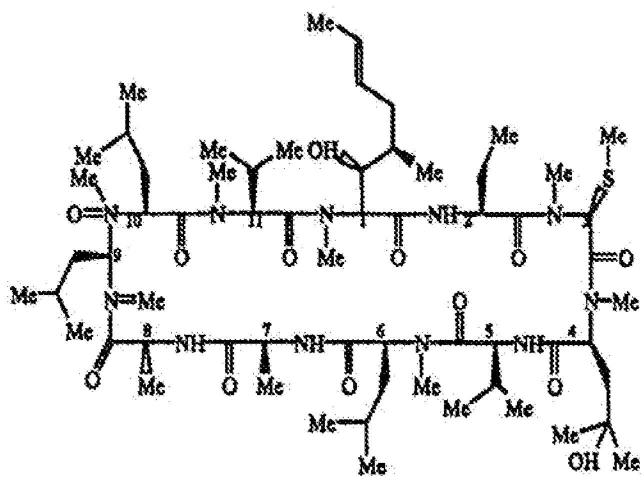
Formula III

(III)



Formula IV

(I)



wherein Me is methyl; Alk is 2-6C alkylene or 3-6C cycloalkylene; R is OH, COOH, alkoxycarbonyl, $-NR_1R_2$ or $N(R_3)-(CH_2)-NR_1R_2$; wherein R_1, R_2 is H, alkyl, 3-6C cycloalkyl, phenyl (optionally substituted by halo, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino), benzyl or saturated or unsaturated heterocyclyl having 5 or 6 members and 1-3 heteroatoms; or NR_1R_2 is a 5 or 6 membered heterocycle which may contain a further N, O or S heteroatom and may be alkylated; R_3 is H or alkyl and n is 2-4; and the alkyl moieties contain 1-4C.

In one embodiment, the cyclosporin component is effective as an immunosuppressant. Without wishing to be limited to any particular theory of operation, it is believed that, in certain embodiments of the present invention, the cyclosporin component acts to enhance or restore lacrimal gland tearing in providing the desired

therapeutic effect.

One important feature of the present invention is that the presently useful compositions contain less than 0.1% by weight of the cyclosporin component. The advantages of such low-concentrations of cyclosporin components have been discussed in some detail elsewhere herein. Low concentrations of cyclosporin component, together with concentrations of the hydrophobic component such that the weight ratio of cyclosporin component to hydrophobic component is greater than 0.08, provides one or more substantial advantages in the present methods.

Any suitable hydrophobic component may be employed in the present invention. Such hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions, with the water or aqueous phase being considered the continuous phase in such emulsion. The hydrophobic component is preferably selected so as to solubilize the cyclosporin component, which is often substantially insoluble in the aqueous phase. Thus, with a suitable hydrophobic component included in the presently useful emulsions, the cyclosporin component is preferably solubilized in the emulsions.

In one very useful embodiment, the hydrophobic component comprises an oily material, in particular, a material which is substantially not miscible in water. Examples of useful oily materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils, and the like and mixtures thereof. Thus, the present hydrophilic components may comprise naturally occurring oils, including, without limitation refined naturally occurring oils, or naturally occurring oils which

have been processed to alter their chemical structures to some extent or oils which are substantially entirely synthetic. One very useful hydrophobic component includes higher fatty acid glycerides.

Examples of useful hydrophobic components include, without limitation, olive oil, arachis oil, castor oil, mineral oil, silicone fluid and the like and mixtures thereof. Higher fatty acid glycerides such as olive oil, peanut oil, castor oil and the like and mixtures thereof are particularly useful in the present invention. Excellent results are obtained using a hydrophobic component comprising castor oil. Without wishing to limit the invention to any particular theory of operation, it is believed that castor oil includes a relatively high concentration of ricinoleic acid which itself may be useful in benefitting ocular tissue and/or in providing one or more therapeutic effects when administered to an eye.

The hydrophobic component is preferably present in the presently useful cyclosporin component-containing emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount up to about 0.75% by weight or about 1.0% by weight or about 1.5% by weight or more of the presently useful emulsion compositions.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the present methods and/or the presently useful compositions. Examples of such other components include, without limitation, emulsifier components, surfactant components, tonicity components, poly electrolyte components, emulsion stability components, viscosity inducing components, demulcent components, acid

and/or bases to adjust the pH of the composition, buffer components, preservative components and the like.

In one very useful embodiment, the presently useful compositions are substantially free of preservatives. Thus, the presently useful compositions may be sterilized and maintained in a sterile condition prior to use, for example, provided in a sealed package or otherwise maintained in a substantially sterile condition.

Any suitable emulsifier component may be employed in the presently useful compositions, provided, that such emulsifier component is effective in forming maintaining the emulsion and/or in the hydrophobic component in emulsion, while having no significant or undue detrimental effect or effects on the compositions during storage or use.

In addition, the presently useful compositions, as well as each of the components of the present compositions in the concentration present in the composition advantageously are ophthalmically acceptable.

Useful emulsifier components may be selected from such component which are conventionally used and well known in the art. Examples of such emulsifier components include, without limitation, surface active components or surfactant components which may be anionic, cationic, nonionic or amphorteric in nature. In general, the emulsifier component includes a hydrophobic constituent and a hydrophilic constituent. Advantageously, the emulsifier component is water soluble in the presently useful compositions. Preferably, the emulsifier component is nonionic. Specific examples of suitable emulsifier components include, without limitation, polysorbate 80, polyoxyalkylene alkylene ethers, polyalkylene oxide ethers

of alkyl alcohols, polyalkylene oxide ethers of alkylphenols, other emulsifiers/surfactants, preferably nonionic emulsifiers/surfactants, useful in ophthalmic compositions, and the like and mixtures thereof.

The emulsifier component is present in an amount effective in forming the present emulsion and/or in maintaining the hydrophobic component in emulsion with the water or aqueous component. In one preferred embodiment, the emulsifier component is present in an amount in a range of about 0.1% to about 5%, more preferably about 0.2% to about 2% and still more preferably about 0.5% to about 1.5% by weight of the presently useful compositions.

Polyelectrolyte or emulsion stabilizing components may be included in the presently useful compositions. Such components are believed to be effective in maintaining the electrolyte balance in the presently useful emulsions, thereby stabilizing the emulsions and preventing the emulsions from breaking down prior to use. In one embodiment, the presently useful compositions include a polyanionic component effective as an emulsion stabilizing component. Examples of suitable polyanionic components useful in the presently useful compositions include, without limitation, anionic cellulose derivatives, anionic acrylic acid-containing polymers, anionic methacrylic acid-containing polymers, anionic amino acid-containing polymers and the like and mixtures thereof.

A particularly useful class of polyanionic components include one or more polymeric materials having multiple anionic charges. Examples include, but are not limited to:

metal carboxy methylcelluloses
metal carboxy methylhydroxyethylcelluloses

metal carboxy methylstarchs
metal carboxy methylhydroxyethylstarchs
hydrolyzed polyacrylamides and polyacrylonitriles
heparin
gucoaminoglycans
hyaluronic acid
chondroitin sulfate
dermatan sulfate
peptides and polypeptides
alginic acid
metal alginates
homopolymers and copolymers of one or more of:
    acrylic and methacrylic acids
    metal acrylates and methacrylates
    vinylsulfonic acid
    metal vinylsulfonate
    amino acids, such as aspartic acid, glutamic acid and the like
    metal salts of amino acids
    p-styrenesulfonic acid
    metal p-styrenesulfonate
    2-methacryloyloxyethylsulfonic acids
    metal 2-methacryloyloxethylsulfonates
    3-methacryloyloxy-2-hydroxypropylsulonic acids
    metal 3-methacryloyloxy-2-hydroxypropylsulfonates
    2-acrylamido-2-methylpropanesulfonic acids
    metal 2-acrylamido-2-methylpropanesulfonates
    allylsulfonic acid
    metal allylsulfonate and the like.

One particularly useful emulsion stabilizing component

includes crosslinked polyacrylates, such as carbomers and Pemulen® materials. Pemulen® is a registered trademark of B.F. Goodrich for polymeric emulsifiers and are commercially available from B.F. Goodrich Company, Specialty Polymers & Chemicals Division, Cleveland, Ohio. Pemulen® materials include acrylate/C10-30 alkyl acrylate cross-polymers, or high molecular weight co-polymers of acrylic acid and a long chain alkyl methacrylate cross-linked with allyl ethers of pentaerythritol.

The presently useful polyanionic components may also be used to provide a suitable viscosity to the presently useful compositions. Thus, the polyanionic components may be useful in stabilizing the presently useful emulsions and in providing a suitable degree of viscosity to the presently useful compositions.

The polyelectrolyte or emulsion stabilizing component advantageously is present in an amount effective to at least assist in stabilizing the cyclosporin component-containing emulsion. For example, the polyelectrolyte/emulsion stabilizing component may be present in an amount in a range of about 0.01% by weight or less to about 1% by weight or more, preferably about 0.02% by weight to about 0.5% by weight, of the composition.

Any suitable tonicity component may be employed in accordance with the present invention. Preferably, such tonicity component is non-ionic, for example, in order to avoid interfering with the other components in the presently useful emulsions and to facilitate maintaining the stability of the emulsion prior to use. Useful tonicity agents include, without limitation, glycerine, mannitol, sorbitol and the like and mixtures thereof. The presently useful emulsions are preferably within the range

of plus or minus about 20% or about 10% from being isotonic.

Ophthalmic demulcent components may be included in effective amounts in the presently useful compositions. For example, ophthalmic demulcent components such as carboxymethylcellulose, other cellulose polymers, dextran 70, gelatin, glycerine, polyethylene glycols (e.g., PEG 300 and PEG 400), polysorbate 80, propylene glycol, polyvinyl alcohol, povidone and the like and mixtures thereof, may be used in the present ophthalmic compositions, for example, compositions useful for treating dry eye.

The demulcent components are preferably present in the compositions, for example, in the form of eye drops, in an amount effective in enhancing the lubricity of the presently useful compositions. The amount of demulcent component in the present compositions may be in a range of at least about 0.01% or about 0.02% to about 0.5% or about 1.0% by weight of the composition.

Many of the presently useful polyelectrolyte/emulsion stabilizing components may also be effective as demulcent components, and vice versa. The emulsifier/surfactant components may also be effective as demulcent components and vice versa.

The pH of the emulsions can be adjusted in a conventional manner using sodium hydroxide and/or hydrochloric acid to a physiological pH level. The pH of the presently useful emulsions preferably is in the range of about 6 to about 10, more preferably about 7.0 to about 8.0 and still more preferably about 7.2 to about 7.6.

Although buffer components are not required in the presently useful compositions, suitable buffer components, for example, and without limitation, phosphates, citrates,

acetates, borates and the like and mixtures thereof, may be employed to maintain a suitable pH in the presently useful compositions.

The presently useful compositions may include an effective amount of a preservative component. Any suitable preservative or combination of preservatives may be employed. Examples of suitable preservatives include, without limitation, benzalkonium chloride, methyl and ethyl parabens, hexetidine, phenyl mercuric salts and the like and mixtures thereof. The amounts of preservative components included in the present compositions are such to be effective in preserving the compositions and can vary based on the specific preservative component employed, the specific composition involved, the specific application involved, and the like factors. Preservative concentrations often are in the range of about 0.00001% to about 0.05% or about 0.1% (w/v) of the composition, although other concentrations of certain preservatives may be employed.

Very useful examples of preservative components in the present invention include, but are not limited to, chlorite components. Specific examples of chlorite components useful as preservatives in accordance with the present invention include stabilized chlorine dioxide (SCD), metal chlorites such as alkali metal and alkaline earth metal chlorites, and the like and mixtures thereof. Technical grade (or USP grade) sodium chlorite is a very useful preservative component. The exact chemical composition of many chlorite components, for example, SCD, is not completely understood. The manufacture or production of certain chlorite components is described in McNicholas U.S. Patent 3,278,447, which is incorporated in its entirety by

reference herein. Specific examples of useful SCD products include that sold under the trademark Dura Klor by Rio Linda Chemical Company, Inc., and that sold under the trademark Anthium Dioxide® by International Dioxide, Inc. An especially useful SCD is a product sold under the trademark Bio-Cide® by Bio-Cide International, Inc., as well as a product identified by Allergan, Inc. by the trademark Purite®.

Other useful preservatives include antimicrobial peptides. Among the antimicrobial peptides which may be employed include, without limitation, defensins, peptides related to defensins, cecropins, peptides related to cecropins, magainins and peptides related to magainins and other amino acid polymers with antibacterial, antifungal and/or antiviral activities. Mixtures of antimicrobial peptides or mixtures of antimicrobial peptides with other preservatives are also included within the scope of the present invention.

The compositions of the present invention may include viscosity modifying agents or components, such as cellulose polymers, including hydroxypropyl methyl cellulose (HPMC), hydroxyethyl cellulose (HEC), ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose and carboxymethyl cellulose; carbomers (e.g. carbopol, and the like); polyvinyl alcohol; polyvinyl pyrrolidone; alginates; carrageenans; and guar, karaya, agarose, locust bean, tragacanth and xanthan gums. Such viscosity modifying components are employed, if at all, in an amount effective to provide a desired viscosity to the present compositions. The concentration of such viscosity modifiers will typically vary between about 0.01 to about 5 % w/v of the

total composition, although other concentrations of certain viscosity modifying components may be employed.

The presently useful compositions may be produced using conventional and well known methods useful in producing ophthalmic products including oil-in-water emulsions.

In one example, the oily phase of the emulsion can be combined with the cyclosporin component to solubilize the cyclosporin component in the oily material phase. The oily phase and the water may be separately heated to an appropriate temperature. This temperature may be the same in both cases, generally a few degrees to about 10°C above the melting temperature of the ingredient(s) having the highest melting point in the case of a solid or semi-solid oily phase for emulsifier components in the oily phase. Where the oily phase is a liquid at room temperature, a suitable temperature for preparation of a composition may be determined by routine experimentation in which the melting point of the ingredients aside from the oily phase is determined. In cases where all components of either the oily phase or the water phase are soluble at room temperature, no heating may be necessary. Non-emulsifying agents which are water soluble are dissolved in the water and oil soluble components including the surfactant components are dissolved in the oily phase.

To create an oil-in-water emulsion, the final oil phase is gently mixed into either an intermediate, preferably de-ionized water, phase or into the final water phase to create a suitable dispersion and the product is allowed to cool with or without stirring. In the case where the final oil phase is first gently mixed into an intermediate water phase, the resulting emulsion

concentrate is thereafter mixed in the appropriate ratio with the final aqueous phase. In such cases, the emulsion concentrate and the final aqueous phase may not be at the same temperature or heated above room temperature, as the emulsion may be already formed at this point.

The oil-in-water emulsions of the present invention can be sterilized after preparation using heat, for example, autoclave steam sterilization or can be sterile filtered using, for example, a 0.22 micron sterile filter. Sterilization employing a sterilization filter can be used when the emulsion droplet (or globule or particle) size and characteristics allows this. The droplet size distribution of the emulsion need not be entirely below the particle size cutoff of the 0.22 micron sterile filtration membrane to be sterile-filtratable. In cases wherein the droplet size distribution of the emulsion is above the particle size cutoff of the 0.22 micron sterile filtration membrane, the emulsion needs to be able to deform or change while passing through the filtration membrane and then reform after passing through. This property is easily determined by routine testing of emulsion droplet size distributions and percent of total oil in the compositions before and after filtration. Alternatively, a loss of a small amount of larger droplet sized material may be acceptable.

The present oil-in-water emulsions preferably are thermodynamicaly stable, much like microemulsions, and yet may not be isotropic transparent compositions as are microemulsions. The emulsions of the present invention advantageously have a shelf life exceeding one year at room temperature.

The following non-limiting examples illustrate certain aspects of the present invention.

EXAMPLE 1

Two compositions are selected for testing. These compositions are produced in accordance with well known techniques and have the following make-ups:

	Composition I	Composition II
	wt%	wt%
Cyclosporin A	0.1	0.05
Castor Oil	1.25	1.25
Polysorbate 80	1.00	1.00
Premulen®	0.05	0.05
Glycerine	2.20	2.20
Sodium hydroxide	qs	qs
Purified Water	qs	qs
pH	7.2-7.6	7.2-7.6
Weight Ratio of Cyclosporin A to Castor Oil	0.08	0.04

These compositions are employed in a Phase 3, double-masked, randomized, parallel group study for the treatment of dry eye disease.

The results of this study indicate that Composition II, in accordance with the present invention, which has a reduced concentration of cyclosporin A and a cyclosporin A to castor oil ratio of less than 0.08, provides overall efficacy in treating dry eye disease substantially equal to that of Composition I. This is surprising for a number of reasons. For example, the reduced concentration of cyclosporin A in Composition II would have been expected to result in reduced overall efficacy in treating dry eye disease. Also, the large amount of castor oil relative to the amount of cyclosporin A in Composition II might have been expected to cause increased eye irritation relative to

Composition I. However, both Composition I and Composition II are found to be substantially non-irritating in use.

Using relatively increased amounts of castor oil, with reduced amounts of cyclosporin component, as in Composition II, is believed to take advantage of the benefits, for example the ocular lubrication benefits, of castor oil, as well as the presence of ricinoleic acid in the castor oil, to at least assist in treating dry eye syndrome in combination with cyclosporin A.

In addition, it is found that the high concentration of castor oil relative to cyclosporin component, as in Composition II, provides the advantage of more quickly or rapidly (for example, relative to a composition which includes only 50% as much castor oil) breaking down or resolving the emulsion in the eye, for example, as measured by split-lamp techniques to monitor the composition in the eye for phase separation. Such rapid break down of the emulsion in the eye reduces vision distortion as the result of the presence of the emulsion in the eye, as well as facilitating the therapeutic effectiveness of the composition in treating dry eye disease.

Using reduced amounts of cyclosporin A, as in Composition II, to achieve therapeutic effectiveness mitigates even further against undesirable side effects and potential drug interactions. Prescribing physicians can provide (prescribe) Composition II to more patients and/or with fewer restrictions and/or with reduced risk of the occurrence of adverse events, e.g., side effects, drug interactions and the like, relative to providing Composition I.

While this invention has been described with respect to various specific examples and embodiments, it is to be

understood that the invention is not limited thereto and that it can be variously practiced within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating an eye of a human or animal comprising:

administering to an eye of a human or animal a composition in the form of an emulsion comprising water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the composition, the weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

2. The method of claim 1 wherein the administering step is effective in treating a condition selected from the group consisting of dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic keratoconjunctivitis and corneal graft rejection.

3. The method of claim 1 wherein the administering step is effective in treating dry eye syndrome.

4. The method of claim 1 wherein the blood of the human or animal has substantially no detectable concentration of the cyclosporin component.

5. The method of claim 1 wherein the blood of the human or animal has substantially no detectable concentration of the cyclosporin component as measured using a validated liquid chromatography/mass spectrometry-mass spectrometry analytical method.

6. The method of claim 1 wherein the blood of the human or animal has a concentration of the cyclosporin component of 0.1 ng/ml or less.

7. The method of claim 1 wherein the cyclosporin component comprises a material selected from cyclosporin A, derivatives of cyclosporin A and mixtures thereof.

8. The method of claim 1 wherein the cyclosporin component comprises cyclosporin A.

9. The method of claim 1 wherein the cyclosporin component is solubilized in the hydrophobic component present in the composition.

10. The method of claim 1 wherein the hydrophobic component is present in the composition in an amount greater than 0.625% by weight of the composition.

11. The method of claim 1 wherein the hydrophobic component comprises an oily material.

12. The method of claim 1 wherein the hydrophobic component comprises an ingredient selected from the group consisting of vegetable oils, animal oils, mineral oils, synthetic oils and mixtures thereof.

13. The method of claim 1 wherein the hydrophobic component comprises castor oil.

14. The method of claim 1 wherein the administering step comprises topically administering the composition to the eye of the human.

15. The method of claim 1 wherein the composition comprises an effective amount of an emulsifier component.

16. The method of claim 1 wherein the composition comprises an effective amount of a tonicity component.

17. The method of claim 1 wherein the composition comprises an effective amount of an organic tonicity component.

18. The method of claim 1 wherein the composition comprises a polyelectrolyte component in an amount effective in stabilizing the composition.

19. The method of claim 1 wherein the composition has a pH in the range of about 7.0 to about 8.0.

20. The method of claim 1 wherein the composition has a pH in the range of about 7.2 to about 7.6.

21. A composition for treating an eye of a human or animal comprising an emulsion comprising water, a hydrophobic component, and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight, the weight ratio of the cyclosporin component to the hydrophobic component being less than 0.08.

22. The composition of claim 21 having a make-up so that when the composition is administered to an eye of a

human in an effective amount in treating dry eye syndrome, the blood of the human has substantially no detectable concentration of the cyclosporin component.

23. The composition of claim 21 wherein the cyclosporin component comprises a material selected from cyclosporin A, derivatives of cyclosporin A and mixtures thereof.

24. The composition of claim 21 wherein the cyclosporin component comprises cyclosporin A.

25. The composition of claim 21 in the form of an emulsion.

26. The composition of claim 21 wherein the hydrophobic component is present in an amount greater than 0.625% by weight of the composition.

27. The composition of claim 21 wherein the hydrophobic component is an oily material.

28. The composition of claim 21 wherein the hydrophobic component comprises an ingredient selected from the group consisting of vegetable oils, animal oils, mineral oils, synthetic oils, and mixtures thereof.

29. The composition of claim 21 wherein the hydrophobic component comprises castor oil.

30. The composition of claim 21 wherein the administering step comprises topically administering the composition to the eye of the human.

31. The composition of claim 21 wherein the composition comprises an effective amount of an emulsifier component.

32. The composition of claim 21 wherein the composition comprises an effective amount of a tonicity component.

33. The composition of claim 21 wherein the composition comprises an effective amount of an organic tonicity component.

34. The composition of claim 21 wherein the composition comprises a polyelectrolytic component in an amount effective in stabilizing the composition.

35. The composition of claim 21 wherein the composition includes water and has a pH in the range of about 7.0 to about 8.0.

36. The composition of claim 21 wherein the composition includes water and has a pH in the range of about 7.2 to about 7.6.

METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Abstract of the Disclosure

Methods of treating an eye of a human or animal include administering to an eye of a human or animal a composition in the form of an emulsion including water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the composition. The weight ratio of the cyclosporin component to the hydrophobic component is less than 0.8.

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Application Number	unknown
Filing Date	herewith
First Named Inventor	Andrew Acheampong
Title	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
Art Unit	
Examiner Name	
Attorney Docket Number	17618CON6B (AP)

SIGNATURE of Applicant or Patent Practitioner

Signature	/Laura L. Wine/	Date	August 14, 2013
Name	Laura L. Wine	Telephone	714-246-6996
Registration Number	68,681		

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications.

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This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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I am the Applicant:

[ ] Inventor or Joint Inventor

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (*e.g.*, a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document)

SIGNATURE of Applicant for Patent

Signature	D Condino	Date	
Name	Debra D. Condino, Reg. No. 31,007	Telephone	714-246-2388
Title and Company	Assistant Secretary, Allergan, Inc.		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms for more than one signature, see below *.

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/967,163	Filing Date 08/14/2013	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	08/14/2013	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 25	Minus	** 25	= 0	x $80 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	x $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LDRC
/NICOLE NICHOLSON/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Acheampong, *et al.*	Examiner: TBA
Serial No.: 13/967,163	Group Art Unit: 1629
Filed: August 14, 2013	Confirmation No. 4274
For: METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS	Customer No.: 51957

SUBMISSION OF SUBSTITUTE SPECIFICATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

The Applicants file with this paper 1) a substitute specification, marked to show changes against the specification filed on August 14, 2013; and 2) a clean version of the specification, incorporating those changes, in compliance with 37 CFR 1.125(c). The applicants have revised the specification to show the changes made by the preliminary amendment filed on August14, 2013; they have not added any new matter. Please replace the specification (excluding the claims) of the above-referenced application with the substitute specification.

As stated in the preliminary amendment filed on August 14, 2013, support for the amendment to the specification at page 4, line 25 – page 5, line 3 of the specification filed August 14, 2013, which corresponds to page 3, line 26 – page 4, line 4 of the substitute and clean specifications filed herewith, may be found, at least, in U.S. Patent Nos. 5,474,979 and 6,254,860, which were previously incorporated by reference in the present application specification at page 1, lines 18-21. The amendment contains no new matter.

The Commissioner is hereby authorized to charge any fees required or necessary for the filing, processing or entering of this paper or any of the enclosed papers, and to refund any overpayment, to deposit account 01-0885.

Respectfully submitted,

/Laura L. Wine/

Date: August 26, 2013

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METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Related Application

This application is a continuation of copending U.S. Application Serial No. 13/961,828 filed August 7, 2013, which is a continuation of copending U.S. Application Serial No. 11/897,177, filed August 28, 2007, which is a continuation of U.S. Application Serial No. 10/927,857, filed August 27, 2004, now abandoned, which claimed the benefit of U.S. Provisional Application No. 60/503,137 filed September 15, 2003, which are incorporated in their entirety herein by reference.

Background of the Invention

The present invention relates to methods of providing desired therapeutic effects to humans or animals using compositions including cyclosporin components. More particularly, the invention relates to methods including administering to an eye of a human or animal a therapeutically effective amount of a cyclosporin component to provide a desired therapeutic effect, preferably a desired ophthalmic or ocular therapeutic effect.

The use of cyclosporin-A and cyclosporin A derivatives to treat ophthalmic conditions has been the subject of various patents, for example Ding et al U.S. Patent 5,474,979; Garst U.S. Patent 6,254,860; and Garst U.S. 6,350,442, this disclosure of each of which is incorporated in its entirely herein by reference. In addition, cyclosporin A compositions used in treating ophthalmic conditions is the subject of a number of publications. Such publications include, for example, "Blood concentrations of cyclosporin a during long-term treatment with cyclosporin a ophthalmic emulsions in patients with moderate to severe dry eye disease," Small et al, *J Ocul Pharmacol Ther, 2002 Oct, 18(5):411-8*; "Distribution of cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs," Acheampong et al, *Curr Eye Res, 1999 Feb, 18(2):91-103b*; "Cyclosporine distribution into the conjunctiva, cornea, lacrimal gland, and systemic blood following topical dosing of cyclosporine to rabbit, dog, and human eyes," Acheampong et al, *Adv Exp Med Biol, 1998, 438:1001-4*; "Preclinical safety studies of cyclosporine ophthalmic emulsion," Angelov et al, *Adv Exp Med Biol, 1998, 438:991-5*; "Cyclosporin & Emulsion & Eye," Stevenson et al, *Ophthalmology, 2000 May, 107(5):967-74*;

and "Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group," Sall et al, *Ophthalmology, 2000 Apr, 107(4):631-9*. Each of these publications is incorporated in its entirety herein by reference. In addition, cyclosporin A-containing oil-in-water emulsions have been clinically tested, under conditions of confidentiality, since the mid 1990's in order to obtain U.S. Food and Drug Administration (FDA) regulatory approval.

Examples of useful cyclosporin A-containing emulsions are set out in Ding et al U.S. Patent 5,474,979. Example 1 of this patent shows a series of emulsions in which the ratio of cyclosporin A to castor oil in each of these compositions was 0.08 or greater, except for Composition B, which included 0.2% by weight cyclosporin A and 5% by weight castor oil. The Ding et al patent placed no significance in Composition B relative to Compositions A, C and D of Example 1.

Over time, it has become apparent that cyclosporin A emulsions for ophthalmic use preferably have less than 0.2% by weight of cyclosporin A. With cyclosporin A concentrations less than 0.2%, the amount of castor oil employed has been reduced since one of the functions of the castor oil is to solubilize the cyclosporin A. Thus, if reduced amounts of cyclosporin are employed, reduced amounts of castor oil are needed to provide effective solubilization of cyclosporin A.

There continues to be a need for providing enhanced methods of treating ophthalmic or ocular conditions with cyclosporin-containing emulsions.

Summary of the Invention

New methods of treating a human or animal using cyclosporin component-containing emulsions have been discovered. Such methods provide substantial overall efficacy in providing desired therapeutic effects. In addition, other important benefits are obtained employing the present methods. For example, patient safety is enhanced. In particular, the present methods provide for reduced risks of side effects and/or drug interactions. Prescribing physicians advantageously have increased flexibility in prescribing such methods and the compositions useful in such methods, for example, because of the reduced risks of harmful side effects and/or drug interactions. The present methods can be easily practiced. In short, the present methods provide substantial and acceptable overall efficacy, together with other advantages, such as

increased safety and/or flexibility.

In one aspect of the present invention, the present methods comprise administering to an eye of a human or animal a composition in the form of an emulsion comprising water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the composition. The weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

It has been found that the relatively increased amounts of hydrophobic component together with relatively reduced, yet therapeutically effective, amounts of cyclosporin component provide substantial and advantageous benefits. For example, the overall efficacy of the present compositions, for example in treating dry eye disease, is substantially equal to an identical composition in which the cyclosporin component is present in an amount of 0.1% by weight. Further, a relatively high concentration of hydrophobic component is believed to provide for a more quick or rapid breaking down or resolving of the emulsion in the eye, which reduces vision distortion which may be caused by the presence of the emulsion in the eye and/or facilitates the therapeutic effectiveness of the composition. Additionally, and importantly, using reduced amounts of the active cyclosporin component mitigates against undesirable side effects and/or potential drug interactions.

In short, the present invention provides at least one advantageous benefit, and preferably a plurality of advantageous benefits.

The present methods are useful in treating any suitable condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome. Cyclosporin has been found as effective in treating immune mediated keratoconjunctivitis sicca (KCS or dry eye disease) in a patient suffering therefrom. The activity of cyclosporins is as an immunosuppressant and in the enhancement or restoring of lacrimal gland tearing. Other conditions that can be treated with cyclosporin components include an absolute or partial deficiency in aqueous tear production (keratoconjunctivitis sicca, or KCS). Topical administration to a patient's tear deficient eye can

increase tear production in the eye. The treatment can further serve to correct corneal and conjunctival disorders exacerbated by tear deficiency and KCS, such as corneal scarring, corneal ulceration, inflammation of the cornea or conjunctiva, filamentary keratisis, mucopurulent discharge and vascularization of the cornea.

Employing reduced concentrations of cyclosporin component, as in the present invention, is advantageously effective to provide the blood of the human or animal under treatment with reduced concentrations of cyclosporin component, preferably with substantially no detectable concentration of the cyclosporin component. The cyclosporin component concentration of blood can be advantageously measured using a validated liquid chromatography/mass spectrometry-mass spectrometry (VLC/MS-MS) analytical method, such as described elsewhere herein.

In one embodiment, in the present methods the blood of the human or animal has concentrations of clyclosporin component of 0.1 ng/ml or less.

Any suitable cyclosporin component effective in the present methods may be used.

Cyclosporins are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. Cyclosporin A, along with several other minor metabolites, cyclosporin B through I, have been identified. In addition, a number of synthetic analogs have been prepared.

In general, commercially available cyclosporins may contain a mixture of several individual cyclosporins which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different substituents or configurations of some of the amino acids.

The term “cyclosporin component” as used herein is intended to include any individual member of the cyclosporin group and derivatives thereof, as well as mixtures of two or more individual cyclosporins and derivatives thereof.

Particularly preferred cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof. Cyclosporin A is an especially useful cyclosporin component.

Any suitable hydrophobic component may be employed in the present invention. Advantageously, the cyclosporin component is solubilized in the hydrophobic component. The hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions.

The hydrophobic component preferably is present in the emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount of up to about 1.0% by weight or about 1.5% by weight or more of the composition.

Preferably, the hydrophobic component comprises one or more oily materials. Examples of useful oil materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils and the like and mixtures thereof. In a very useful embodiment, the hydrophobic component comprises one or more higher fatty acid glycerides. Excellent results are obtained when the hydrophobic component comprises castor oil.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the compositions. Examples of such other components include, without limitation, emulsifier components, tonicity components, polyelectrolyte components, surfactant components, viscosity inducing components, acids and/or bases to adjust the pH of the composition, buffer components, preservative components and the like. Components may be employed which are effective to perform two or more functions in the presently useful compositions. For example, components which are effective as both emulsifiers and surfactants may be employed, and/or components which are effective as both polyelectrolyte components and viscosity inducing components may be employed. The specific composition chosen for use in the present invention advantageously is selected taking into account various factors present in the specific application at hand, for example, the desired therapeutic effect to be achieved, the desired properties of the compositions to be employed, the sensitivities of the human or animal to whom the composition is to be administered, and the like factors.

The presently useful compositions advantageously are ophthalmically acceptable. A composition, component or material is ophthalmically acceptable when it is compatible with ocular tissue, that is, it does not cause significant or undue detrimental effects when brought into contact with ocular tissues.

Such compositions have pH’s within the physiological range of about 6 to about 10, preferably in a range of about 7.0 to about 8.0 and more preferably in a range of about 7.2 to about 7.6.

The present methods preferably provide for an administering step comprising topically administering the presently useful compositions to the eye or eyes of a human or animal.

Each and every feature described herein, and each and every combination of two or more of such features, is included within the scope of the present invention provided that the features included in such a combination are not mutually inconsistent.

These and other aspects and advantages of the present invention are apparent in the following detailed description, example and claims.

Detailed Description

The present methods are effective for treating an eye of a human or animal. Such methods, in general, comprise administering, preferably topically administering, to an eye of a human or animal a cyclosporin component-containing emulsion. The emulsion contains water, for example U.S. pure water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the emulsion. In addition, beneficial results have been found when the weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

As noted above, the present administering step preferably includes topically administering the emulsion to the eye of a patient of a human or animal. Such administering may involve a single use of the presently useful compositions, or repeated or periodic use of such compositions, for example, as required or desired to achieve the therapeutic effect to be obtained. The topical administration of the presently useful composition may involve providing the composition in the form of eye drops or similar form or other form so as to facilitate such topical administration.

The present methods have been found to be very effective in providing the desired therapeutic effect or effects while, at the same time, substantially reducing, or even substantially eliminating, side effects which may result from the presence of the cyclosporin component in the blood of the human or animal being treated, and eye irritation which, in the past, has been caused by the presence of certain components in prior art cyclosporin-containing emulsions. Also, the use of the present compositions which include reduced amounts of the cyclosporin components allow for more frequent administration of the present compositions to achieve the desired therapeutic effect or effects without substantially increasing the risk of side effects and/or eye irritation.

The present methods are useful in treating any condition which is therapeutically

sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome.

The frequency of administration and the amount of the presently useful composition to use during each administration varies depending upon the therapeutic effect to be obtained, the severity of the condition being treated and the like factors. The presently useful compositions are designed to allow the prescribing physician substantial flexibility in treating various ocular conditions to achieve the desired therapeutic effect or effects with reduced risk of side effects and/or eye irritation. Such administration may occur on an as needed basis, for example, in treating or managing dry eye syndrome, on a one time basis or on a repeated or periodic basis once, twice, thrice or more times daily depending on the needs of the human or animal being treated and other factors involved in the application at hand.

One of the important advantages of the present invention is the reduced concentration of the cyclosporin component in the blood of the human or animal as a result of administering the present composition as described herein. One very useful embodiment of the present administering step provides no substantial detectable concentration of cyclosporin component in the blood of the human or animal. Cyclosporin component concentration in blood preferably is determined using a liquid chromatography-mass spectroscopy-mass spectroscopy (LC-MS/MS), which test has a cyclosporin component detection limit of 0.1 ng/ml. Cyclosporin component concentrations below or less than 0.1 ng/ml are therefore considered substantially undetectable.

The LC-MS/MS test is advantageously run as follows.

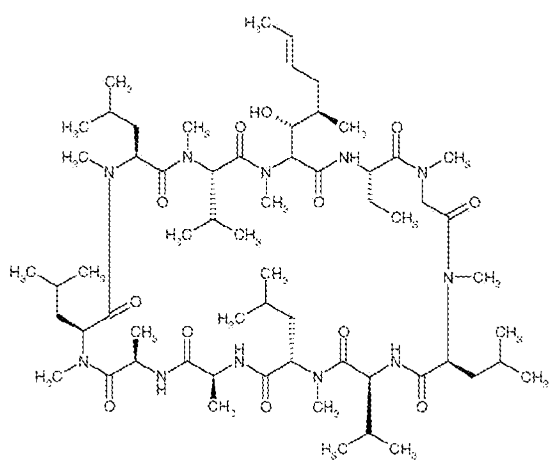
One ml of blood is acidified with 0.2 ml of 0.1 N HCl solution, then extracted with 5 ml of methyl t-butyl ether. After separation from the acidified aqueous layer, the organic phase is neutralized with 2 ml of 0.1 N NaOH, evaporated, reconstituted in a water/acetonitrile-based mobil phase, and injected onto a 2.1 x 50 mm, 3µm pore size C-8 reverse phase high pressure liquid chromatography (HPLC) column (Keystone Scientific, Bellefonte, PA). Compounds are gradient-eluted at 0.2 mL/min and detected using an API III triple quadrupole mass spectrometer with a turbo-ionspray source (PE-Sciex, Concord, Ontario, Canada). Molecular reaction

monitoring enhances the sensitivity and selectivity of this assay. Protonated molecules for the analyte and an internal standard are collisionally dissociated and product ions at m/z 425 are monitored for the analyte and the internal standard. Under these conditions, cyclosporin A and the internal standard cyclosporin G elute with retention times of about 3.8 minutes. The lower limit of quantitation is 0.1 ng/mL, at which concentration the coefficient of variation and deviation from nominal concentration is <15%.

As noted previously, any suitable cyclosporin component effective in the present methods may be employed. Very useful cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof.

The chemical structure for cyclosporin A is represented by Formula 1

Formula 1



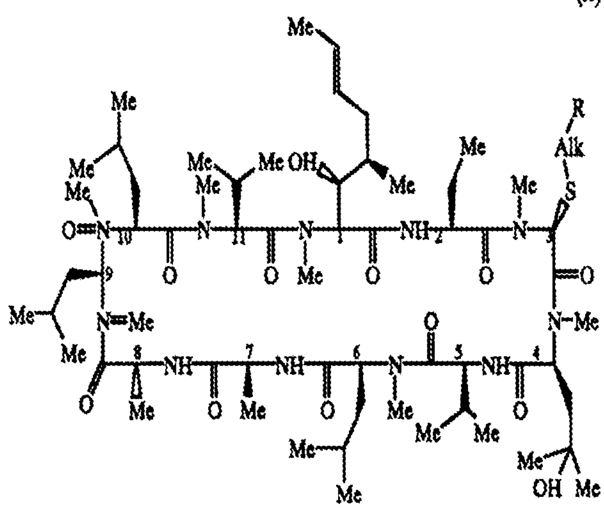
As used herein the term "derivatives" of a cyclosporin refer to compounds having structures sufficiently similar to the cyclosporin so as to function in a manner substantially similar to or substantially identical to the cyclosporin, for example, cyclosporin A, in the present methods. Included, without limitation, within the useful cyclosporin A derivatives are those selected from ((R)-methylthio-Sar)^3-(4'-hydroxy-MeLeu) cyclosporin A, ((R)-(Cyclo)alkylthio-Sar)^3-(4'-hydroxy-MeLeu)^4-cyclosporin A, and ((R)-(Cyclo)alkylthio-Sar)^3-cyclosporin A derivatives described below.

These cyclosporin derivatives are represented by the following general formulas (II),

(III), and (IV) respectively:

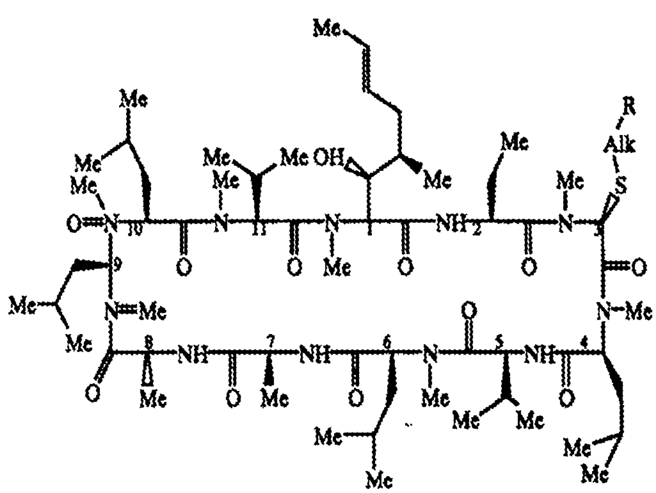
Formula II

(II)



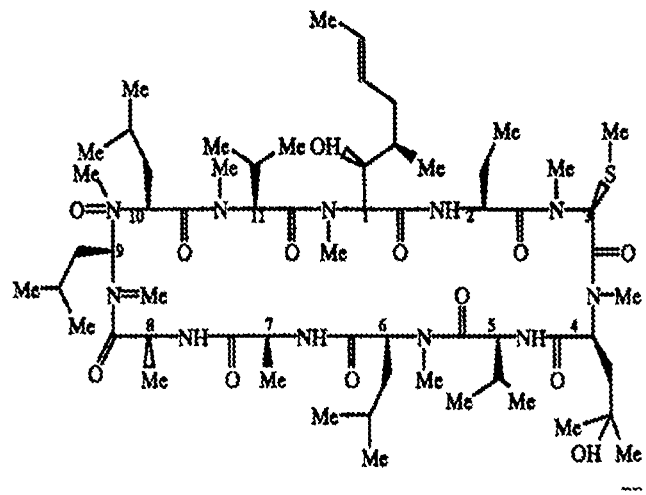
Formula III

(III)



Formula IV

(I)



wherein Me is methyl; Alk is 2-6C alkylene or 3-6C cycloalkylene; R is OH, COOH, alkoxycarbonyl, $-NR_1R_2$ or $N(R_3)C(CH_2)CNR_1R_2$; wherein R_1,R_2 is H, alkyl, 3-6C cycloalkyl, phenyl (optionally substituted by halo, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino), benzyl or saturated or unsaturated heterocyclyl having 5 or 6 members and 1-3 heteroatoms; or NR_1R_2 is a 5 or 6 membered heterocycle which may contain a further N, O or S heteroatom and may be alkylated; R_3 is H or alkyl and n is 2-4; and the alkyl moieties contain 1-4C.

In one embodiment, the cyclosporin component is effective as an immunosuppressant. Without wishing to be limited to any particular theory of operation, it is believed that, in certain embodiments of the present invention, the cyclosporin component acts to enhance or restore lacrimal gland tearing in providing the desired therapeutic effect.

One important feature of the present invention is that the presently useful compositions contain less than 0.1% by weight of the cyclosporin component. The advantages of such low-concentrations of cyclosporin components have been discussed in some detail elsewhere herein. Low concentrations of cyclosporin component, together with concentrations of the hydrophobic component such that the weight ratio of cyclosporin component to hydrophobic component is greater than 0.08, provides one or more substantial advantages in the present methods.

Any suitable hydrophobic component may be employed in the present invention. Such hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions, with the water or aqueous phase being

considered the continuous phase in such emulsion. The hydrophobic component is preferably selected so as to solubilize the cyclosporin component, which is often substantially insoluble in the aqueous phase. Thus, with a suitable hydrophobic component included in the presently useful emulsions, the cyclosporin component is preferably solubilized in the emulsions.

In one very useful embodiment, the hydrophobic component comprises an oily material, in particular, a material which is substantially not miscible in water. Examples of useful oily materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils, and the like and mixtures thereof. Thus, the present hydrophilic components may comprise naturally occurring oils, including, without limitation refined naturally occurring oils, or naturally occurring oils which have been processed to alter their chemical structures to some extent or oils which are substantially entirely synthetic. One very useful hydrophobic component includes higher fatty acid glycerides.

Examples of useful hydrophobic components include, without limitation, olive oil, arachis oil, castor oil, mineral oil, silicone fluid and the like and mixtures thereof. Higher fatty acid glycerides such as olive oil, peanut oil, castor oil and the like and mixtures thereof are particularly useful in the present invention. Excellent results are obtained using a hydrophobic component comprising castor oil. Without wishing to limit the invention to any particular theory of operation, it is believed that castor oil includes a relatively high concentration of ricinoleic acid which itself may be useful in benefitting ocular tissue and/or in providing one or more therapeutic effects when administered to an eye.

The hydrophobic component is preferably present in the presently useful cyclosporin component-containing emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount up to about 0.75% by weight or about 1.0% by weight or about 1.5% by weight or more of the presently useful emulsion compositions.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the present methods and/or the presently useful compositions. Examples of such other components include, without limitation, emulsifier components, surfactant components, tonicity components, poly electrolyte components, emulsion stability components, viscosity inducing components, demulcent components, acid and/or bases to adjust the pH of the composition, buffer components,

preservative components and the like.

In one very useful embodiment, the presently useful compositions are substantially free of preservatives. Thus, the presently useful compositions may be sterilized and maintained in a sterile condition prior to use, for example, provided in a sealed package or otherwise maintained in a substantially sterile condition.

Any suitable emulsifier component may be employed in the presently useful compositions, provided, that such emulsifier component is effective in forming maintaining the emulsion and/or in the hydrophobic component in emulsion, while having no significant or undue detrimental effect or effects on the compositions during storage or use.

In addition, the presently useful compositions, as well as each of the components of the present compositions in the concentration present in the composition advantageously are ophthalmically acceptable.

Useful emulsifier components may be selected from such component which are conventionally used and well known in the art. Examples of such emulsifier components include, without limitation, surface active components or surfactant components which may be anionic, cationic, nonionic or amphorteric in nature. In general, the emulsifier component includes a hydrophobic constituent and a hydrophilic constituent. Advantageously, the emulsifier component is water soluble in the presently useful compositions. Preferably, the emulsifier component is nonionic. Specific examples of suitable emulsifier components include, without limitation, polysorbate 80, polyoxyalkylene alkylene ethers, polyalkylene oxide ethers of alkyl alcohols, polyalkylene oxide ethers of alkylphenols, other emulsifiers/surfactants, preferably nonionic emulsifiers/surfactants, useful in ophthalmic compositions, and the like and mixtures thereof.

The emulsifier component is present in an amount effective in forming the present emulsion and/or in maintaining the hydrophobic component in emulsion with the water or aqueous component. In one preferred embodiment, the emulsifier component is present in an amount in a range of about 0.1% to about 5%, more preferably about 0.2% to about 2% and still more preferably about 0.5% to about 1.5% by weight of the presently useful compositions.

Polyelectrolyte or emulsion stabilizing components may be included in the presently useful compositions. Such components are believed to be effective in maintaining the electrolyte balance in the presently useful emulsions, thereby stabilizing the emulsions and preventing the

emulsions from breaking down prior to use. In one embodiment, the presently useful compositions include a polyanionic component effective as an emulsion stabilizing component. Examples of suitable polyanionic components useful in the presently useful compositions include, without limitation, anionic cellulose derivatives, anionic acrylic acid-containing polymers, anionic methacrylic acid-containing polymers, anionic amino acid-containing polymers and the like and mixtures thereof.

A particularly useful class of polyanionic components include one or more polymeric materials having multiple anionic charges. Examples include, but are not limited to:

metal carboxy methylcelluloses
metal carboxy methylhydroxyethylcelluloses
metal carboxy methylstarchs
metal carboxy methylhydroxyethylstarchs
hydrolyzed polyacrylamides and polyacrylonitriles
heparin
gucoaminoglycans
hyaluronic acid
chondroitin sulfate
dermatan sulfate
peptides and polypeptides
alginic acid
metal alginates
homopolymers and copolymers of one or more of:
    acrylic and methacrylic acids
    metal acrylates and methacrylates
    vinylsulfonic acid
    metal vinylsulfonate
    amino acids, such as aspartic acid, glutamic acid and the like
    metal salts of amino acids
    p-styrenesulfonic acid
    metal p-styrenesulfonate

2-methacryloyloxyethylsulfonic acids
metal 2-methacryloyloxethylsulfonates
3-methacryloyloxy-2-hydroxypropylsulonic acids
metal 3-methacryloyloxy-2-
hydroxypropylsulfonates
2-acrylamido-2-methylpropanesulfonic acids
metal 2-acrylamido-2-methylpropanesulfonates
allylsulfonic acid
metal allylsulfonate and the like.

One particularly useful emulsion stabilizing component includes crosslinked polyacrylates, such as carbomers and Pemulen® materials. Pemulen® is a registered trademark of B.F. Goodrich for polymeric emulsifiers and are commercially available from B.F. Goodrich Company, Specialty Polymers & Chemicals Division, Cleveland, Ohio. Pemulen® materials include acrylate/C10-30 alkyl acrylate cross-polymers, or high molecular weight co-polymers of acrylic acid and a long chain alkyl methacrylate cross-linked with allyl ethers of pentaerythritol.

The presently useful polyanionic components may also be used to provide a suitable viscosity to the presently useful compositions. Thus, the polyanionic components may be useful in stabilizing the presently useful emulsions and in providing a suitable degree of viscosity to the presently useful compositions.

The polyelectrolyte or emulsion stabilizing component advantageously is present in an amount effective to at least assist in stabilizing the cyclosporin component-containing emulsion. For example, the polyelectrolyte/emulsion stabilizing component may be present in an amount in a range of about 0.01% by weight or less to about 1% by weight or more, preferably about 0.02% by weight to about 0.5% by weight, of the composition.

Any suitable tonicity component may be employed in accordance with the present invention. Preferably, such tonicity component is non-ionic, for example, in order to avoid interfering with the other components in the presently useful emulsions and to facilitate maintaining the stability of the emulsion prior to use. Useful tonicity agents include, without limitation, glycerine, mannitol, sorbitol and the like and mixtures thereof. The presently useful emulsions are preferably within the range of plus or minus about 20% or about 10% from being

isotonic.

Ophthalmic demulcent components may be included in effective amounts in the presently useful compositions. For example, ophthalmic demulcent components such as carboxymethylcellulose, other cellulose polymers, dextran 70, gelatin, glycerine, polyethylene glycols (e.g., PEG 300 and PEG 400), polysorbate 80, propylene glycol, polyvinyl alcohol, povidone and the like and mixtures thereof, may be used in the present ophthalmic compositions, for example, compositions useful for treating dry eye.

The demulcent components are preferably present in the compositions, for example, in the form of eye drops, in an amount effective in enhancing the lubricity of the presently useful compositions. The amount of demulcent component in the present compositions may be in a range of at least about 0.01% or about 0.02% to about 0.5% or about 1.0% by weight of the composition.

Many of the presently useful polyelectrolyte/emulsion stabilizing components may also be effective as demulcent components, and vice versa. The emulsifier/surfactant components may also be effective as demulcent components and vice versa.

The pH of the emulsions can be adjusted in a conventional manner using sodium hydroxide and/or hydrochloric acid to a physiological pH level. The pH of the presently useful emulsions preferably is in the range of about 6 to about 10, more preferably about 7.0 to about 8.0 and still more preferably about 7.2 to about 7.6.

Although buffer components are not required in the presently useful compositions, suitable buffer components, for example, and without limitation, phosphates, citrates, acetates, borates and the like and mixtures thereof, may be employed to maintain a suitable pH in the presently useful compositions.

The presently useful compositions may include an effective amount of a preservative component. Any suitable preservative or combination of preservatives may be employed. Examples of suitable preservatives include, without limitation, benzalkonium chloride, methyl and ethyl parabens, hexetidine, phenyl mercuric salts and the like and mixtures thereof. The amounts of preservative components included in the present compositions are such to be effective in preserving the compositions and can vary based on the specific preservative component employed, the specific composition involved, the specific application involved, and the like factors. Preservative concentrations often are in the range of about 0.00001% to about

0.05% or about 0.1% (w/v) of the composition, although other concentrations of certain preservatives may be employed.

Very useful examples of preservative components in the present invention include, but are not limited to, chlorite components. Specific examples of chlorite components useful as preservatives in accordance with the present invention include stabilized chlorine dioxide (SCD), metal chlorites such as alkali metal and alkaline earth metal chlorites, and the like and mixtures thereof. Technical grade (or USP grade) sodium chlorite is a very useful preservative component. The exact chemical composition of many chlorite components, for example, SCD, is not completely understood. The manufacture or production of certain chlorite components is described in McNicholas U.S. Patent 3,278,447, which is incorporated in its entirety by reference herein. Specific examples of useful SCD products include that sold under the trademark Dura Klor by Rio Linda Chemical Company, Inc., and that sold under the trademark Anthium Dioxide® by International Dioxide, Inc. An especially useful SCD is a product sold under the trademark Bio-Cide® by Bio-Cide International, Inc., as well as a product identified by Allergan, Inc. by the trademark Purite®.

Other useful preservatives include antimicrobial peptides. Among the antimicrobial peptides which may be employed include, without limitation, defensins, peptides related to defensins, cecropins, peptides related to cecropins, magainins and peptides related to magainins and other amino acid polymers with antibacterial, antifungal and/or antiviral activities. Mixtures of antimicrobial peptides or mixtures of antimicrobial peptides with other preservatives are also included within the scope of the present invention.

The compositions of the present invention may include viscosity modifying agents or components, such as cellulose polymers, including hydroxypropyl methyl cellulose (HPMC), hydroxyethyl cellulose (HEC), ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose and carboxymethyl cellulose; carbomers (e.g. carbopol, and the like); polyvinyl alcohol; polyvinyl pyrrolidone; alginates; carrageenans; and guar, karaya, agarose, locust bean, tragacanth and xanthan gums. Such viscosity modifying components are employed, if at all, in an amount effective to provide a desired viscosity to the present compositions. The concentration of such viscosity modifiers will typically vary between about 0.01 to about 5 % w/v of the total composition, although other concentrations of certain viscosity modifying components may be employed.

The presently useful compositions may be produced using conventional and well known methods useful in producing ophthalmic products including oil-in-water emulsions.

In one example, the oily phase of the emulsion can be combined with the cyclosporin component to solubilize the cyclosporin component in the oily material phase. The oily phase and the water may be separately heated to an appropriate temperature. This temperature may be the same in both cases, generally a few degrees to about 10°C above the melting temperature of the ingredient(s) having the highest melting point in the case of a solid or semi-solid oily phase for emulsifier components in the oily phase. Where the oily phase is a liquid at room temperature, a suitable temperature for preparation of a composition may be determined by routine experimentation in which the melting point of the ingredients aside from the oily phase is determined. In cases where all components of either the oily phase or the water phase are soluble at room temperature, no heating may be necessary. Non-emulsifying agents which are water soluble are dissolved in the water and oil soluble components including the surfactant components are dissolved in the oily phase.

To create an oil-in-water emulsion, the final oil phase is gently mixed into either an intermediate, preferably de-ionized water, phase or into the final water phase to create a suitable dispersion and the product is allowed to cool with or without stirring. In the case where the final oil phase is first gently mixed into an intermediate water phase, the resulting emulsion concentrate is thereafter mixed in the appropriate ratio with the final aqueous phase. In such cases, the emulsion concentrate and the final aqueous phase may not be at the same temperature or heated above room temperature, as the emulsion may be already formed at this point.

The oil-in-water emulsions of the present invention can be sterilized after preparation using heat, for example, autoclave steam sterilization or can be sterile filtered using, for example, a 0.22 micron sterile filter. Sterilization employing a sterilization filter can be used when the emulsion droplet (or globule or particle) size and characteristics allows this. The droplet size distribution of the emulsion need not be entirely below the particle size cutoff of the 0.22 micron sterile filtration membrane to be sterile-filtratable. In cases wherein the droplet size distribution of the emulsion is above the particle size cutoff of the 0.22 micron sterile filtration membrane, the emulsion needs to be able to deform or change while passing through the filtration membrane and then reform after passing through. This property is easily determined by routine testing of emulsion droplet size distributions and percent of total oil in the compositions before and after

filtration. Alternatively, a loss of a small amount of larger droplet sized material may be acceptable.

The present oil-in-water emulsions preferably are thermodynamicaly stable, much like microemulsions, and yet may not be isotropic transparent compositions as are microemulsions. The emulsions of the present invention advantageously have a shelf life exceeding one year at room temperature.

The following non-limiting examples illustrate certain aspects of the present invention.

EXAMPLE 1

Two compositions are selected for testing. These compositions are produced in accordance with well known techniques and have the following make-ups:

	Composition I wt%	Composition II wt%
Cyclosporin	0.1	0.05
Castor Oil	1.25	1.25
Polysorbate 80	1.00	1.00
Premulen®	0.05	0.05
Glycerine	2.20	2.20
Sodium hydroxide	qs	qs
Purified Water	qs	qs
pH	7.2-7.6	7.2-7.6
Weight Ratio of Cyclosporin A to Castor Oil	0.08	0.04

These compositions are employed in a Phase 3, double-masked, randomized, parallel group study for the treatment of dry eye disease.

The results of this study indicate that Composition II, in accordance with the present invention, which has a reduced concentration of cyclosporin A and a cyclosporin A to castor oil ratio of less than 0.08, provides overall efficacy in treating dry eye disease substantially equal to that of Composition I. This is surprising for a number of reasons. For example, the reduced concentration of cyclosporin A in Composition II would have been expected to result in reduced overall efficacy in treating dry eye disease. Also, the large amount of castor oil relative to the

amount of cyclosporin A in Composition II might have been expected to cause increased eye irritation relative to Composition I. However, both Composition I and Composition II are found to be substantially non-irritating in use.

Using relatively increased amounts of castor oil, with reduced amounts of cyclosporin component, as in Composition II, is believed to take advantage of the benefits, for example the ocular lubrication benefits, of castor oil, as well as the presence of ricinoleic acid in the castor oil, to at least assist in treating dry eye syndrome in combination with cyclosporin A.

In addition, it is found that the high concentration of castor oil relative to cyclosporin component, as in Composition II, provides the advantage of more quickly or rapidly (for example, relative to a composition which includes only 50% as much castor oil) breaking down or resolving the emulsion in the eye, for example, as measured by split-lamp techniques to monitor the composition in the eye for phase separation. Such rapid break down of the emulsion in the eye reduces vision distortion as the result of the presence of the emulsion in the eye, as well as facilitating the therapeutic effectiveness of the composition in treating dry eye disease.

Using reduced amounts of cyclosporin A, as in Composition II, to achieve therapeutic effectiveness mitigates even further against undesirable side effects and potential drug interactions. Prescribing physicians can provide (prescribe) Composition II to more patients and/or with fewer restrictions and/or with reduced risk of the occurrence of adverse events, e.g., side effects, drug interactions and the like, relative to providing Composition I.

While this invention has been described with respect to various specific examples and embodiments, it is to be understood that the invention is not limited thereto and that it can be variously practiced within the scope of the following claims.

METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Related Application

This application is a continuation of copending U.S. Application Serial No. 13/961,828 filed August 7, 2013, which is a continuation of copending U.S. Application Serial No. 11/897,177, filed August 28, 2007, which is a continuation of U.S. Application Serial No. 10/927,857, filed August 27, 2004, now abandoned, which claimed the benefit of U.S. Provisional Application No. 60/503,137 filed September 15, 2003, which ~~is~~ are incorporated in ~~its~~ their entirety herein by reference.

Background of the Invention

The present invention relates to methods of providing desired therapeutic effects to humans or animals using compositions including cyclosporin components. More particularly, the invention relates to methods including administering to an eye of a human or animal a therapeutically effective amount of a cyclosporin component to provide a desired therapeutic effect, preferably a desired ophthalmic or ocular therapeutic effect.

The use of cyclosporin-A and cyclosporin A derivatives to treat ophthalmic conditions has been the subject of various patents, for example Ding et al U.S. Patent 5,474,979; Garst U.S. Patent 6,254,860; and Garst U.S. 6,350,442, this disclosure of each of which is incorporated in its entirely herein by reference. In addition, cyclosporin A compositions used in treating ophthalmic conditions is the subject of a number of publications. Such publications include, for example, "Blood concentrations of cyclosporin a during long-term treatment with cyclosporin a ophthalmic emulsions in patients with moderate to severe dry eye disease," Small et al, *J Ocul Pharmacol Ther, 2002 Oct, 18(5):411-8*; "Distribution of cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs," Acheampong et al, *Curr Eye Res, 1999 Feb, 18(2):91-103b*; "Cyclosporine distribution into the conjunctiva, cornea, lacrimal gland, and systemic blood following topical dosing of cyclosporine to rabbit, dog, and human eyes," Acheampong et al, *Adv Exp Med Biol, 1998, 438:1001-4*; "Preclinical safety studies of cyclosporine ophthalmic emulsion," Angelov et al, *Adv Exp Med Biol, 1998, 438:991-5*; "Cyclosporin & Emulsion & Eye," Stevenson et al, *Ophthalmology, 2000 May, 107(5):967-74*;

and "Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group," Sall et al, *Ophthalmology, 2000 Apr, 107(4):631-9*. Each of these publications is incorporated in its entirety herein by reference. In addition, cyclosporin A-containing oil-in-water emulsions have been clinically tested, under conditions of confidentiality, since the mid 1990's in order to obtain U.S. Food and Drug Administration (FDA) regulatory approval.

Examples of useful cyclosporin A-containing emulsions are set out in Ding et al U.S. Patent 5,474,979. Example 1 of this patent shows a series of emulsions in which the ratio of cyclosporin A to castor oil in each of these compositions was 0.08 or greater, except for Composition B, which included 0.2% by weight cyclosporin A and 5% by weight castor oil. The Ding et al patent placed no significance in Composition B relative to Compositions A, C and D of Example 1.

Over time, it has become apparent that cyclosporin A emulsions for ophthalmic use preferably have less than 0.2% by weight of cyclosporin A. With cyclosporin A concentrations less than 0.2%, the amount of castor oil employed has been reduced since one of the functions of the castor oil is to solubilize the cyclosporin A. Thus, if reduced amounts of cyclosporin are employed, reduced amounts of castor oil are needed to provide effective solubilization of cyclosporin A.

There continues to be a need for providing enhanced methods of treating ophthalmic or ocular conditions with cyclosporin-containing emulsions.

Summary of the Invention

New methods of treating a human or animal using cyclosporin component-containing emulsions have been discovered. Such methods provide substantial overall efficacy in providing desired therapeutic effects. In addition, other important benefits are obtained employing the present methods. For example, patient safety is enhanced. In particular, the present methods provide for reduced risks of side effects and/or drug interactions. Prescribing physicians advantageously have increased flexibility in prescribing such methods and the compositions useful in such methods, for example, because of the reduced risks of harmful side effects and/or drug interactions. The present methods can be easily practiced. In short, the present methods provide substantial and acceptable overall efficacy, together with other advantages, such as

increased safety and/or flexibility.

In one aspect of the present invention, the present methods comprise administering to an eye of a human or animal a composition in the form of an emulsion comprising water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the composition. The weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

It has been found that the relatively increased amounts of hydrophobic component together with relatively reduced, yet therapeutically effective, amounts of cyclosporin component provide substantial and advantageous benefits. For example, the overall efficacy of the present compositions, for example in treating dry eye disease, is substantially equal to an identical composition in which the cyclosporin component is present in an amount of 0.1% by weight. Further, a relatively high concentration of hydrophobic component is believed to provide for a more quick or rapid breaking down or resolving of the emulsion in the eye, which reduces vision distortion which may be caused by the presence of the emulsion in the eye and/or facilitates the therapeutic effectiveness of the composition. Additionally, and importantly, using reduced amounts of the active cyclosporin component mitigates against undesirable side effects and/or potential drug interactions.

In short, the present invention provides at least one advantageous benefit, and preferably a plurality of advantageous benefits.

The present methods are useful in treating any suitable condition which is therapeutically sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome. Cyclosporin has been found as effective in treating immune mediated keratoconjunctivitis sicca (KCS or dry eye disease) in a patient suffering therefrom. The activity of cyclosporins is as an immunosuppressant and in the enhancement or restoring of lacrimal gland tearing. Other conditions that can be treated with cyclosporin components include an absolute or partial deficiency in aqueous tear production (keratoconjunctivitis sicca, or KCS). Topical administration to a patient's tear deficient eye can

increase tear production in the eye. The treatment can further serve to correct corneal and conjunctival disorders exacerbated by tear deficiency and KCS, such as corneal scarring, corneal ulceration, inflammation of the cornea or conjunctiva, filamentary keratisis, mucopurulent discharge and vascularization of the cornea.

Employing reduced concentrations of cyclosporin component, as in the present invention, is advantageously effective to provide the blood of the human or animal under treatment with reduced concentrations of cyclosporin component, preferably with substantially no detectable concentration of the cyclosporin component. The cyclosporin component concentration of blood can be advantageously measured using a validated liquid chromatography/mass spectrometry-mass spectrometry (VLC/MS-MS) analytical method, such as described elsewhere herein.

In one embodiment, in the present methods the blood of the human or animal has concentrations of clyclosporin component of 0.1 ng/ml or less.

Any suitable cyclosporin component effective in the present methods may be used.

Cyclosporins are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. Cyclosporin A, along with several other minor metabolites, cyclosporin B through I, have been identified. In addition, a number of synthetic analogs have been prepared.

In general, commercially available cyclosporins may contain a mixture of several individual cyclosporins which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different substituents or configurations of some of the amino acids.

The term “cyclosporin component” as used herein is intended to include any individual member of the cyclosporin group and derivatives thereof, as well as mixtures of two or more individual cyclosporins and derivatives thereof.

Particularly preferred cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof. Cyclosporin A is an especially useful cyclosporin component.

Any suitable hydrophobic component may be employed in the present invention. Advantageously, the cyclosporin component is solubilized in the hydrophobic component. The hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions.

The hydrophobic component preferably is present in the emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount of up to about 1.0% by weight or about 1.5% by weight or more of the composition.

Preferably, the hydrophobic component comprises one or more oily materials. Examples of useful oil materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils and the like and mixtures thereof. In a very useful embodiment, the hydrophobic component comprises one or more higher fatty acid glycerides. Excellent results are obtained when the hydrophobic component comprises castor oil.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the compositions. Examples of such other components include, without limitation, emulsifier components, tonicity components, polyelectrolyte components, surfactant components, viscosity inducing components, acids and/or bases to adjust the pH of the composition, buffer components, preservative components and the like. Components may be employed which are effective to perform two or more functions in the presently useful compositions. For example, components which are effective as both emulsifiers and surfactants may be employed, and/or components which are effective as both polyelectrolyte components and viscosity inducing components may be employed. The specific composition chosen for use in the present invention advantageously is selected taking into account various factors present in the specific application at hand, for example, the desired therapeutic effect to be achieved, the desired properties of the compositions to be employed, the sensitivities of the human or animal to whom the composition is to be administered, and the like factors.

The presently useful compositions advantageously are ophthalmically acceptable. A composition, component or material is ophthalmically acceptable when it is compatible with ocular tissue, that is, it does not cause significant or undue detrimental effects when brought into contact with ocular tissues.

Such compositions have pH's within the physiological range of about 6 to about 10, preferably in a range of about 7.0 to about 8.0 and more preferably in a range of about 7.2 to about 7.6.

The present methods preferably provide for an administering step comprising topically administering the presently useful compositions to the eye or eyes of a human or animal.

Each and every feature described herein, and each and every combination of two or more of such features, is included within the scope of the present invention provided that the features included in such a combination are not mutually inconsistent.

These and other aspects and advantages of the present invention are apparent in the following detailed description, example and claims.

Detailed Description

The present methods are effective for treating an eye of a human or animal. Such methods, in general, comprise administering, preferably topically administering, to an eye of a human or animal a cyclosporin component-containing emulsion. The emulsion contains water, for example U.S. pure water, a hydrophobic component and a cyclosporin component in a therapeutically effective amount of less than 0.1% by weight of the emulsion. In addition, beneficial results have been found when the weight ratio of the cyclosporin component to the hydrophobic component is less than 0.08.

As noted above, the present administering step preferably includes topically administering the emulsion to the eye of a patient of a human or animal. Such administering may involve a single use of the presently useful compositions, or repeated or periodic use of such compositions, for example, as required or desired to achieve the therapeutic effect to be obtained. The topical administration of the presently useful composition may involve providing the composition in the form of eye drops or similar form or other form so as to facilitate such topical administration.

The present methods have been found to be very effective in providing the desired therapeutic effect or effects while, at the same time, substantially reducing, or even substantially eliminating, side effects which may result from the presence of the cyclosporin component in the blood of the human or animal being treated, and eye irritation which, in the past, has been caused by the presence of certain components in prior art cyclosporin-containing emulsions. Also, the use of the present compositions which include reduced amounts of the cyclosporin components allow for more frequent administration of the present compositions to achieve the desired therapeutic effect or effects without substantially increasing the risk of side effects and/or eye irritation.

The present methods are useful in treating any condition which is therapeutically

sensitive to or treatable with cyclosporin components. Such conditions preferably are ophthalmic or ocular conditions, that is relating to or having to do with one or more parts of an eye of a human or animal. Included among such conditions are, without limitation, dry eye syndrome, phacoanaphylactic endophthalmitis, uveitis, vernal conjunctivitis, atopic kerapoconjunctivitis, corneal graft rejection and the like conditions. The present invention is particularly effective in treating dry eye syndrome.

The frequency of administration and the amount of the presently useful composition to use during each administration varies depending upon the therapeutic effect to be obtained, the severity of the condition being treated and the like factors. The presently useful compositions are designed to allow the prescribing physician substantial flexibility in treating various ocular conditions to achieve the desired therapeutic effect or effects with reduced risk of side effects and/or eye irritation. Such administration may occur on an as needed basis, for example, in treating or managing dry eye syndrome, on a one time basis or on a repeated or periodic basis once, twice, thrice or more times daily depending on the needs of the human or animal being treated and other factors involved in the application at hand.

One of the important advantages of the present invention is the reduced concentration of the cyclosporin component in the blood of the human or animal as a result of administering the present composition as described herein. One very useful embodiment of the present administering step provides no substantial detectable concentration of cyclosporin component in the blood of the human or animal. Cyclosporin component concentration in blood preferably is determined using a liquid chromatography-mass spectroscopy-mass spectroscopy (LC-MS/MS), which test has a cyclosporin component detection limit of 0.1 ng/ml. Cyclosporin component concentrations below or less than 0.1 ng/ml are therefore considered substantially undetectable.

The LC-MS/MS test is advantageously run as follows.

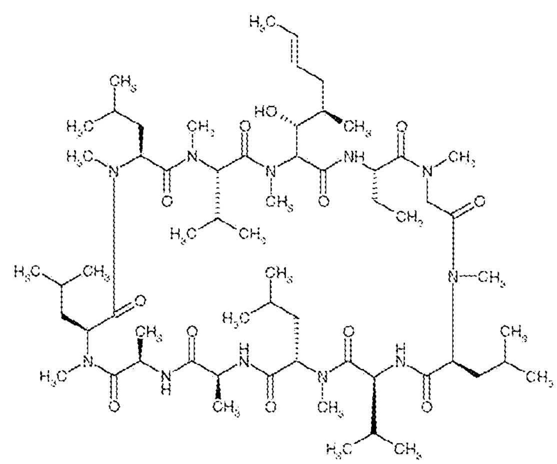
One ml of blood is acidified with 0.2 ml of 0.1 N HCl solution, then extracted with 5 ml of methyl t-butyl ether. After separation from the acidified aqueous layer, the organic phase is neutralized with 2 ml of 0.1 N NaOH, evaporated, reconstituted in a water/acetonitrile-based mobil phase, and injected onto a 2.1 x 50 mm, 3µm pore size C-8 reverse phase high pressure liquid chromatography (HPLC) column (Keystone Scientific, Bellefonte, PA). Compounds are gradient-eluted at 0.2 mL/min and detected using an API III triple quadrupole mass spectrometer with a turbo-ionspray source (PE-Sciex, Concord, Ontario, Canada). Molecular reaction

monitoring enhances the sensitivity and selectivity of this assay. Protonated molecules for the analyte and an internal standard are collisionally dissociated and product ions at m/z 425 are monitored for the analyte and the internal standard. Under these conditions, cyclosporin A and the internal standard cyclosporin G elute with retention times of about 3.8 minutes. The lower limit of quantitation is 0.1 ng/mL, at which concentration the coefficient of variation and deviation from nominal concentration is <15%.

As noted previously, any suitable cyclosporin component effective in the present methods may be employed. Very useful cyclosporin components include, without limitation, cyclosporin A, derivatives of cyclosporin A and the like and mixtures thereof.

The chemical structure for cyclosporin A is represented by Formula 1

Formula 1



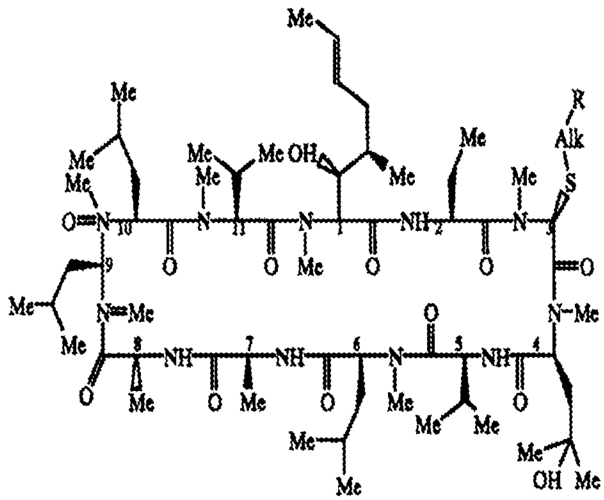
As used herein the term "derivatives" of a cyclosporin refer to compounds having structures sufficiently similar to the cyclosporin so as to function in a manner substantially similar to or substantially identical to the cyclosporin, for example, cyclosporin A, in the present methods. Included, without limitation, within the useful cyclosporin A derivatives are those selected from ((R)-methylthio-Sar)^3-(4'-hydroxy-MeLeu) cyclosporin A, ((R)-(Cyclo)alkylthio-Sar)^3-(4'-hydroxy-MeLeu)^4-cyclosporin A, and ((R)-(Cyclo)alkylthio-Sar)^3-cyclosporin A derivatives described below.

These cyclosporin derivatives are represented by the following general formulas (II),

(III), and (IV) respectively:

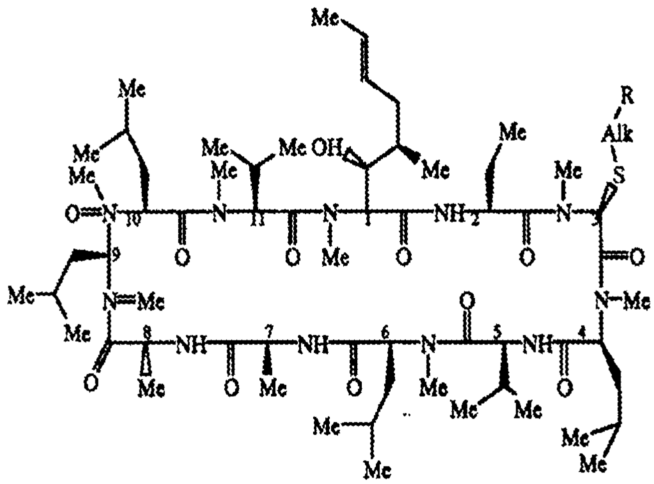
Formula II

(II)



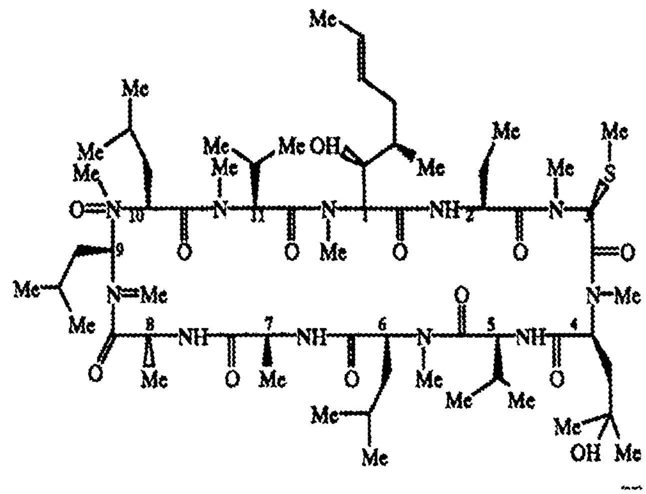
Formula III

(III)



Formula IV

(I)



wherein Me is methyl; Alk is 2-6C alkylene or 3-6C cycloalkylene; R is OH, COOH, alkoxycarbonyl, $-NR_1R_2$ or $N(R_3)C(CH_2)CNR_1R_2$; wherein R_1,R_2 is H, alkyl, 3-6C cycloalkyl, phenyl (optionally substituted by halo, alkoxy, alkoxycarbonyl, amino, alkylamino or dialkylamino), benzyl or saturated or unsaturated heterocyclyl having 5 or 6 members and 1-3 heteroatoms; or NR_1R_2 is a 5 or 6 membered heterocycle which may contain a further N, O or S heteroatom and may be alkylated; R_3 is H or alkyl and n is 2-4; and the alkyl moieties contain 1-4C.

In one embodiment, the cyclosporin component is effective as an immunosuppressant. Without wishing to be limited to any particular theory of operation, it is believed that, in certain embodiments of the present invention, the cyclosporin component acts to enhance or restore lacrimal gland tearing in providing the desired therapeutic effect.

One important feature of the present invention is that the presently useful compositions contain less than 0.1% by weight of the cyclosporin component. The advantages of such low-concentrations of cyclosporin components have been discussed in some detail elsewhere herein. Low concentrations of cyclosporin component, together with concentrations of the hydrophobic component such that the weight ratio of cyclosporin component to hydrophobic component is greater than 0.08, provides one or more substantial advantages in the present methods.

Any suitable hydrophobic component may be employed in the present invention. Such hydrophobic component may be considered as comprising a discontinuous phase in the presently useful cyclosporin component-containing emulsions, with the water or aqueous phase being

considered the continuous phase in such emulsion. The hydrophobic component is preferably selected so as to solubilize the cyclosporin component, which is often substantially insoluble in the aqueous phase. Thus, with a suitable hydrophobic component included in the presently useful emulsions, the cyclosporin component is preferably solubilized in the emulsions.

In one very useful embodiment, the hydrophobic component comprises an oily material, in particular, a material which is substantially not miscible in water. Examples of useful oily materials include, without limitation, vegetable oils, animal oils, mineral oils, synthetic oils, and the like and mixtures thereof. Thus, the present hydrophilic components may comprise naturally occurring oils, including, without limitation refined naturally occurring oils, or naturally occurring oils which have been processed to alter their chemical structures to some extent or oils which are substantially entirely synthetic. One very useful hydrophobic component includes higher fatty acid glycerides.

Examples of useful hydrophobic components include, without limitation, olive oil, arachis oil, castor oil, mineral oil, silicone fluid and the like and mixtures thereof. Higher fatty acid glycerides such as olive oil, peanut oil, castor oil and the like and mixtures thereof are particularly useful in the present invention. Excellent results are obtained using a hydrophobic component comprising castor oil. Without wishing to limit the invention to any particular theory of operation, it is believed that castor oil includes a relatively high concentration of ricinoleic acid which itself may be useful in benefitting ocular tissue and/or in providing one or more therapeutic effects when administered to an eye.

The hydrophobic component is preferably present in the presently useful cyclosporin component-containing emulsion compositions in an amount greater than about 0.625% by weight. For example, the hydrophobic component may be present in an amount up to about 0.75% by weight or about 1.0% by weight or about 1.5% by weight or more of the presently useful emulsion compositions.

The presently useful compositions may include one or more other components in amounts effective to facilitate the usefulness and effectiveness of the present methods and/or the presently useful compositions. Examples of such other components include, without limitation, emulsifier components, surfactant components, tonicity components, poly electrolyte components, emulsion stability components, viscosity inducing components, demulcent components, acid and/or bases to adjust the pH of the composition, buffer components,

preservative components and the like.

In one very useful embodiment, the presently useful compositions are substantially free of preservatives. Thus, the presently useful compositions may be sterilized and maintained in a sterile condition prior to use, for example, provided in a sealed package or otherwise maintained in a substantially sterile condition.

Any suitable emulsifier component may be employed in the presently useful compositions, provided, that such emulsifier component is effective in forming maintaining the emulsion and/or in the hydrophobic component in emulsion, while having no significant or undue detrimental effect or effects on the compositions during storage or use.

In addition, the presently useful compositions, as well as each of the components of the present compositions in the concentration present in the composition advantageously are ophthalmically acceptable.

Useful emulsifier components may be selected from such component which are conventionally used and well known in the art. Examples of such emulsifier components include, without limitation, surface active components or surfactant components which may be anionic, cationic, nonionic or amphorteric in nature. In general, the emulsifier component includes a hydrophobic constituent and a hydrophilic constituent. Advantageously, the emulsifier component is water soluble in the presently useful compositions. Preferably, the emulsifier component is nonionic. Specific examples of suitable emulsifier components include, without limitation, polysorbate 80, polyoxyalkylene alkylene ethers, polyalkylene oxide ethers of alkyl alcohols, polyalkylene oxide ethers of alkylphenols, other emulsifiers/surfactants, preferably nonionic emulsifiers/surfactants, useful in ophthalmic compositions, and the like and mixtures thereof.

The emulsifier component is present in an amount effective in forming the present emulsion and/or in maintaining the hydrophobic component in emulsion with the water or aqueous component. In one preferred embodiment, the emulsifier component is present in an amount in a range of about 0.1% to about 5%, more preferably about 0.2% to about 2% and still more preferably about 0.5% to about 1.5% by weight of the presently useful compositions.

Polyelectrolyte or emulsion stabilizing components may be included in the presently useful compositions. Such components are believed to be effective in maintaining the electrolyte balance in the presently useful emulsions, thereby stabilizing the emulsions and preventing the

emulsions from breaking down prior to use. In one embodiment, the presently useful compositions include a polyanionic component effective as an emulsion stabilizing component. Examples of suitable polyanionic components useful in the presently useful compositions include, without limitation, anionic cellulose derivatives, anionic acrylic acid-containing polymers, anionic methacrylic acid-containing polymers, anionic amino acid-containing polymers and the like and mixtures thereof.

A particularly useful class of polyanionic components include one or more polymeric materials having multiple anionic charges. Examples include, but are not limited to:

metal carboxy methylcelluloses
metal carboxy methylhydroxyethylcelluloses
metal carboxy methylstarchs
metal carboxy methylhydroxyethylstarchs
hydrolyzed polyacrylamides and polyacrylonitriles
heparin
gucoaminoglycans
hyaluronic acid
chondroitin sulfate
dermatan sulfate
peptides and polypeptides
alginic acid
metal alginates
homopolymers and copolymers of one or more of:
    acrylic and methacrylic acids
    metal acrylates and methacrylates
    vinylsulfonic acid
    metal vinylsulfonate
    amino acids, such as aspartic acid, glutamic acid and the like
    metal salts of amino acids
    p-styrenesulfonic acid
    metal p-styrenesulfonate

2-methacryloyloxyethylsulfonic acids
metal 2-methacryloyloxethylsulfonates
3-methacryloyloxy-2-hydroxypropylsulonic acids
metal 3-methacryloyloxy-2-hydroxypropylsulfonates
2-acrylamido-2-methylpropanesulfonic acids
metal 2-acrylamido-2-methylpropanesulfonates
allylsulfonic acid
metal allylsulfonate and the like.

One particularly useful emulsion stabilizing component includes crosslinked polyacrylates, such as carbomers and Pemulen® materials. Pemulen® is a registered trademark of B.F. Goodrich for polymeric emulsifiers and are commercially available from B.F. Goodrich Company, Specialty Polymers & Chemicals Division, Cleveland, Ohio. Pemulen® materials include acrylate/C10-30 alkyl acrylate cross-polymers, or high molecular weight co-polymers of acrylic acid and a long chain alkyl methacrylate cross-linked with allyl ethers of pentaerythritol.

The presently useful polyanionic components may also be used to provide a suitable viscosity to the presently useful compositions. Thus, the polyanionic components may be useful in stabilizing the presently useful emulsions and in providing a suitable degree of viscosity to the presently useful compositions.

The polyelectrolyte or emulsion stabilizing component advantageously is present in an amount effective to at least assist in stabilizing the cyclosporin component-containing emulsion. For example, the polyelectrolyte/emulsion stabilizing component may be present in an amount in a range of about 0.01% by weight or less to about 1% by weight or more, preferably about 0.02% by weight to about 0.5% by weight, of the composition.

Any suitable tonicity component may be employed in accordance with the present invention. Preferably, such tonicity component is non-ionic, for example, in order to avoid interfering with the other components in the presently useful emulsions and to facilitate maintaining the stability of the emulsion prior to use. Useful tonicity agents include, without limitation, glycerine, mannitol, sorbitol and the like and mixtures thereof. The presently useful emulsions are preferably within the range of plus or minus about 20% or about 10% from being

isotonic.

Ophthalmic demulcent components may be included in effective amounts in the presently useful compositions. For example, ophthalmic demulcent components such as carboxymethylcellulose, other cellulose polymers, dextran 70, gelatin, glycerine, polyethylene glycols (e.g., PEG 300 and PEG 400), polysorbate 80, propylene glycol, polyvinyl alcohol, povidone and the like and mixtures thereof, may be used in the present ophthalmic compositions, for example, compositions useful for treating dry eye.

The demulcent components are preferably present in the compositions, for example, in the form of eye drops, in an amount effective in enhancing the lubricity of the presently useful compositions. The amount of demulcent component in the present compositions may be in a range of at least about 0.01% or about 0.02% to about 0.5% or about 1.0% by weight of the composition.

Many of the presently useful polyelectrolyte/emulsion stabilizing components may also be effective as demulcent components, and vice versa. The emulsifier/surfactant components may also be effective as demulcent components and vice versa.

The pH of the emulsions can be adjusted in a conventional manner using sodium hydroxide and/or hydrochloric acid to a physiological pH level. The pH of the presently useful emulsions preferably is in the range of about 6 to about 10, more preferably about 7.0 to about 8.0 and still more preferably about 7.2 to about 7.6.

Although buffer components are not required in the presently useful compositions, suitable buffer components, for example, and without limitation, phosphates, citrates, acetates, borates and the like and mixtures thereof, may be employed to maintain a suitable pH in the presently useful compositions.

The presently useful compositions may include an effective amount of a preservative component. Any suitable preservative or combination of preservatives may be employed. Examples of suitable preservatives include, without limitation, benzalkonium chloride, methyl and ethyl parabens, hexetidine, phenyl mercuric salts and the like and mixtures thereof. The amounts of preservative components included in the present compositions are such to be effective in preserving the compositions and can vary based on the specific preservative component employed, the specific composition involved, the specific application involved, and the like factors. Preservative concentrations often are in the range of about 0.00001% to about

0.05% or about 0.1% (w/v) of the composition, although other concentrations of certain preservatives may be employed.

Very useful examples of preservative components in the present invention include, but are not limited to, chlorite components. Specific examples of chlorite components useful as preservatives in accordance with the present invention include stabilized chlorine dioxide (SCD), metal chlorites such as alkali metal and alkaline earth metal chlorites, and the like and mixtures thereof. Technical grade (or USP grade) sodium chlorite is a very useful preservative component. The exact chemical composition of many chlorite components, for example, SCD, is not completely understood. The manufacture or production of certain chlorite components is described in McNicholas U.S. Patent 3,278,447, which is incorporated in its entirety by reference herein. Specific examples of useful SCD products include that sold under the trademark Dura Klor by Rio Linda Chemical Company, Inc., and that sold under the trademark Anthium Dioxide® by International Dioxide, Inc. An especially useful SCD is a product sold under the trademark Bio-Cide® by Bio-Cide International, Inc., as well as a product identified by Allergan, Inc. by the trademark Purite®.

Other useful preservatives include antimicrobial peptides. Among the antimicrobial peptides which may be employed include, without limitation, defensins, peptides related to defensins, cecropins, peptides related to cecropins, magainins and peptides related to magainins and other amino acid polymers with antibacterial, antifungal and/or antiviral activities. Mixtures of antimicrobial peptides or mixtures of antimicrobial peptides with other preservatives are also included within the scope of the present invention.

The compositions of the present invention may include viscosity modifying agents or components, such as cellulose polymers, including hydroxypropyl methyl cellulose (HPMC), hydroxyethyl cellulose (HEC), ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose and carboxymethyl cellulose; carbomers (e.g. carbopol, and the like); polyvinyl alcohol; polyvinyl pyrrolidone; alginates; carrageenans; and guar, karaya, agarose, locust bean, tragacanth and xanthan gums. Such viscosity modifying components are employed, if at all, in an amount effective to provide a desired viscosity to the present compositions. The concentration of such viscosity modifiers will typically vary between about 0.01 to about 5 % w/v of the total composition, although other concentrations of certain viscosity modifying components may be employed.

The presently useful compositions may be produced using conventional and well known methods useful in producing ophthalmic products including oil-in-water emulsions.

In one example, the oily phase of the emulsion can be combined with the cyclosporin component to solubilize the cyclosporin component in the oily material phase. The oily phase and the water may be separately heated to an appropriate temperature. This temperature may be the same in both cases, generally a few degrees to about 10°C above the melting temperature of the ingredient(s) having the highest melting point in the case of a solid or semi-solid oily phase for emulsifier components in the oily phase. Where the oily phase is a liquid at room temperature, a suitable temperature for preparation of a composition may be determined by routine experimentation in which the melting point of the ingredients aside from the oily phase is determined. In cases where all components of either the oily phase or the water phase are soluble at room temperature, no heating may be necessary. Non-emulsifying agents which are water soluble are dissolved in the water and oil soluble components including the surfactant components are dissolved in the oily phase.

To create an oil-in-water emulsion, the final oil phase is gently mixed into either an intermediate, preferably de-ionized water, phase or into the final water phase to create a suitable dispersion and the product is allowed to cool with or without stirring. In the case where the final oil phase is first gently mixed into an intermediate water phase, the resulting emulsion concentrate is thereafter mixed in the appropriate ratio with the final aqueous phase. In such cases, the emulsion concentrate and the final aqueous phase may not be at the same temperature or heated above room temperature, as the emulsion may be already formed at this point.

The oil-in-water emulsions of the present invention can be sterilized after preparation using heat, for example, autoclave steam sterilization or can be sterile filtered using, for example, a 0.22 micron sterile filter. Sterilization employing a sterilization filter can be used when the emulsion droplet (or globule or particle) size and characteristics allows this. The droplet size distribution of the emulsion need not be entirely below the particle size cutoff of the 0.22 micron sterile filtration membrane to be sterile-filtratable. In cases wherein the droplet size distribution of the emulsion is above the particle size cutoff of the 0.22 micron sterile filtration membrane, the emulsion needs to be able to deform or change while passing through the filtration membrane and then reform after passing through. This property is easily determined by routine testing of emulsion droplet size distributions and percent of total oil in the compositions before and after

filtration. Alternatively, a loss of a small amount of larger droplet sized material may be acceptable.

The present oil-in-water emulsions preferably are thermodynamicaly stable, much like microemulsions, and yet may not be isotropic transparent compositions as are microemulsions. The emulsions of the present invention advantageously have a shelf life exceeding one year at room temperature.

The following non-limiting examples illustrate certain aspects of the present invention.

EXAMPLE 1

Two compositions are selected for testing. These compositions are produced in accordance with well known techniques and have the following make-ups:

	Composition I	Composition II
	wt%	wt%
Cyclosporin	0.1	0.05
Castor Oil	1.25	1.25
Polysorbate 80	1.00	1.00
Premulen®	0.05	0.05
Glycerine	2.20	2.20
Sodium hydroxide	qs	qs
Purified Water	qs	qs
pH	7.2-7.6	7.2-7.6
Weight Ratio of Cyclosporin A to Castor Oil	0.08	0.04

These compositions are employed in a Phase 3, double-masked, randomized, parallel group study for the treatment of dry eye disease.

The results of this study indicate that Composition II, in accordance with the present invention, which has a reduced concentration of cyclosporin A and a cyclosporin A to castor oil ratio of less than 0.08, provides overall efficacy in treating dry eye disease substantially equal to that of Composition I. This is surprising for a number of reasons. For example, the reduced concentration of cyclosporin A in Composition II would have been expected to result in reduced overall efficacy in treating dry eye disease. Also, the large amount of castor oil relative to the

amount of cyclosporin A in Composition II might have been expected to cause increased eye irritation relative to Composition I. However, both Composition I and Composition II are found to be substantially non-irritating in use.

Using relatively increased amounts of castor oil, with reduced amounts of cyclosporin component, as in Composition II, is believed to take advantage of the benefits, for example the ocular lubrication benefits, of castor oil, as well as the presence of ricinoleic acid in the castor oil, to at least assist in treating dry eye syndrome in combination with cyclosporin A.

In addition, it is found that the high concentration of castor oil relative to cyclosporin component, as in Composition II, provides the advantage of more quickly or rapidly (for example, relative to a composition which includes only 50% as much castor oil) breaking down or resolving the emulsion in the eye, for example, as measured by split-lamp techniques to monitor the composition in the eye for phase separation. Such rapid break down of the emulsion in the eye reduces vision distortion as the result of the presence of the emulsion in the eye, as well as facilitating the therapeutic effectiveness of the composition in treating dry eye disease.

Using reduced amounts of cyclosporin A, as in Composition II, to achieve therapeutic effectiveness mitigates even further against undesirable side effects and potential drug interactions. Prescribing physicians can provide (prescribe) Composition II to more patients and/or with fewer restrictions and/or with reduced risk of the occurrence of adverse events, e.g., side effects, drug interactions and the like, relative to providing Composition I.

While this invention has been described with respect to various specific examples and embodiments, it is to be understood that the invention is not limited thereto and that it can be variously practiced within the scope of the following claims.

Electronic Acknowledgement Receipt

EFS ID:	16688246
Application Number:	13967163
International Application Number:	
Confirmation Number:	4274
Title of Invention:	METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS
First Named Inventor/Applicant Name:	Andrew Acheampong
Customer Number:	51957
Filer:	Laura Lee Wine/Bonnie Ferguson
Filer Authorized By:	Laura Lee Wine
Attorney Docket Number:	17618CON6B (AP)
Receipt Date:	26-AUG-2013
Filing Date:	
Time Stamp:	17:02:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	17618CON6BCoverSheet-forSpec.pdf	103562 f4fde7e56027022482b897826a7f0deb7f19b12d	no	2

Warnings:

Information:

2	Specification	17618CON6BNEWCLEANCOPY.pdf	494765 857325721c6bf0c9360fd9ac5ae44abf3e784d19	no	19
Warnings:					
Information:					
3	Specification	17618CON6BNEWMARKEDUPSPEC.pdf	496479 c831f778d92e3ac2824be402427fbab2baa2b1cf	no	19
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			Total Files Size (in bytes):	1094806	

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National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/967,163	08/14/2013	Andrew Acheampong	17618CON6B (AP)

CONFIRMATION NO. 4274

POA ACCEPTANCE LETTER

OC000000063566192

51957
ALLERGAN, INC.
2525 DUPONT DRIVE, T2-7H
IRVINE, CA 92612-1599

Date Mailed: 09/06/2013

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/14/2013.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/nbekele/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/967,163	08/14/2013	1629	2440	17618CON6B (AP)	25	3

CONFIRMATION NO. 4274

51957
ALLERGAN, INC.
2525 DUPONT DRIVE, T2-7H
IRVINE, CA 92612-1599

FILING RECEIPT

OC000000063566144

Date Mailed: 09/06/2013

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Andrew Acheampong, Irvine, CA;
Diane D. Tang-Liu, Las Vegas, NV;
James N. Chang, Newport Beach, CA;
David F. Power, Hubert, NC;

Applicant(s)

Allergan, Inc., Irvine, CA

Assignment For Published Patent Application

Allergan, Inc., Irvine, CA

Power of Attorney: The patent practitioners associated with Customer Number 51957

Domestic Priority data as claimed by applicant

This application is a CON of 13/961,828 08/07/2013
which is a CON of 11/897,177 08/28/2007
and is a CON of 10/927,857 08/27/2004 ABN
which claims benefit of 60/503,137 09/15/2003

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 09/03/2013

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/967,163**

Projected Publication Date: 12/12/2013

Non-Publication Request: No

Early Publication Request: No
Title

METHODS OF PROVIDING THERAPEUTIC EFFECTS USING CYCLOSPORIN COMPONENTS

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number
13/967,163

APPLICATION AS FILED - PART I

FOR	NUMBER FILED (Column 1)	NUMBER EXTRA (Column 2)	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	25 minus 20 =	* 5			OR	x 80 =	400
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
			TOTAL			TOTAL	2000

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED - PART II

AMENDMENT A

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	CLAIMS REMAINING AFTER AMENDMENT (Column 1)		HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2)	PRESENT EXTRA (Column 3)	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/967,163	08/14/2013	Andrew Acheampong	17618CON6B (AP)

CONFIRMATION NO. 4274

51957
ALLERGAN, INC.
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NOTICE

OC000000063566145

Date Mailed: 09/06/2013

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Diane D. Tang-Liu
  Applicant may submit the inventor's oath or declaration at any time before the Notice of Allowance and Fee(s) Due, PTOL-85, is mailed.

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	13967163
	Filing Date	2013-08-14
	First Named Inventor	ACHEAMPONG, ANDREW
	Art Unit	1653
	Examiner Name	TBD
	Attorney Docket Number	17618-US-BCON6-AP

U.S.PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code1	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	3278447		1966-10-11	Thomas McNicholas	
	2	4388229		1983-06-14	Cherng-Chyi Fu	
	3	4388307		1983-06-14	Thomas Cavanak	
	4	4614736		1986-09-30	Delevallee et al	
	5	4649047		1987-03-10	Renee Kaswan	
	6	4764503		1988-08-16	Roland Wenger	
	7	4814323		1989-03-21	Andrieu et al	
	8	4839342		1989-06-13	Renee Kaswan	

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	9	4970076		1990-11-13	David Horrobin	
	10	4990337		1991-02-05	Kurihara et al	
	11	4996193		1991-02-26	Hewitt et al	
	12	5047396		1991-09-10	Orban et al	
	13	5051402		1991-09-24	Kurihara et al	
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	15	5286730		1994-02-15	Caufield et al	
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	20	5368854		1994-11-29	Donna Rennick	
	21	5411952		1995-05-02	Renee Kaswan	
	22	5424078		1995-06-13	Anthony Dziabo	
	23	5474919		1995-12-12	Chartrain et al	
	24	5474979		1995-12-12	Ding et al	U.S. Application No. 08/243,279 and its entire prosecution history**
	25	5504068		1996-04-02	Komiya et al	
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	31	5639724		1997-06-17	Thomas Cavanak	
	32	5652212		1997-07-29	Cavanak et al	
	33	5719123		1998-02-17	Morley et al	
	34	5739105		1998-04-14	Kim et al	
	35	5753166		1998-05-19	Dalton et al	
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	37	5798333		1998-08-25	Bernard Sherman	
	38	5807820		1998-09-15	Elias et al	
	39	5827822		1998-10-27	Floch'h et al	
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	Attorney Docket Number	17618-US-BCON6-AP

	42	5843452		1998-12-01	Wiedmann et al	
	43	5843891		1998-12-01	Bernard Sherman	
	44	5858401		1999-01-12	Bhalani et al	
	45	5866159		1999-02-02	Hauer et al	
	46	5891846		1999-04-06	Ishida et al	
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	48	5929030		1999-07-27	Hamied et al	
	49	5951971		1999-09-14	Kawashima et al	
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	Attorney Docket Number	17618-US-BCON6-AP

	53	5977066		1999-11-02	Thomas Cavanak	
	54	5981479		1999-11-09	Ko et al	
	55	5981607		1999-11-09	Ding et al	U.S. Application No. 09/008,924 and its entire prosecution history**
	56	5998365		1999-12-07	Bernard Sherman	
	57	6004566		1999-12-21	Friedman et al	
	58	6007840		1999-12-28	Hauer et al	
	59	6008191		1999-12-28	Amarjit Singh	
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	64	6057289		2000-05-02	Nirmal Mulye	
	65	6159933		2000-12-12	Bernard Sherman	
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	67	6254860		2001-07-03	Michael Garst	
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	71	6294192		2001-09-25	Patel et al	
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	Attorney Docket Number	17618-US-BCON6-AP

	75	6350442		2002-02-26	Michael Garst	
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Attorney Docket Number	17618-US-BCON6-AP

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	92	7501393		2009-03-10	Tien et al	U.S. Application No. 11/161,218 and its entire prosecution history**
	93	8211855		2012-07-03	Chang et al	U.S. Application No. 11/857,223 and its entire prosecution history**
	94	8288348		2012-10-16	Chang et al	U.S. Application No. 11/917,448 and its entire prosecution history**

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear

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	Attorney Docket Number	17618-US-BCON6-AP

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	2	20010014665		2001-08-16	Fischer et al	
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	10	20020107183		2002-08-08	Petszulat et al	
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	Attorney Docket Number	17618-US-BCON6-AP

	12	20020165134		2002-11-07	Richter et al	
	13	20030021816		2003-01-30	Kang et al	
	14	20030044452		2003-03-06	Ryuji Ueno	
	15	20030055028		2003-03-20	Stergiopoulos et al	
	16	20030059470		2003-03-27	Rainer Muller	
	17	20030060402		2003-03-27	Cavanak et al	
	18	20030087813		2003-05-08	Or et al	
	19	20030104992		2003-06-05	Or et al	
	20	20030108626		2003-06-12	Benita et al	
	21	20030109425		2003-06-12	Or et al	
	22	20030109426		2003-06-12	Or et al	

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	23	20030133984		2003-07-17	Ambuhl et al	
	24	20030143250		2003-07-31	Hauer et al	
	25	20030147954		2003-08-07	Yang et al	
	26	20030166517		2003-09-04	Fricker et al	
	27	20050014691		2005-01-20	Bakhit et al	
	28	20050059583		2005-03-17	Andrew Acheampong	U.S. Application No. 10/927,857 and its entire prosecution history**
	29	20070015691		2007-01-18	James Chang	U.S. Application No. 11/181,409 and its entire prosecution history**
	30	20070027072		2007-02-01	Tien et al	
	31	20070087962		2007-04-19	Tien et al	
	32	20070149447		2007-06-28	Chang et al	U.S. Application No. 11/679,934 and its entire prosecution history**
	33	20070299004		2007-12-27	Acheampong et al	U.S. Application No. 11/897,177 and its entire prosecution history**

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	Attorney Docket Number	17618-US-BCON6-AP

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	First Named Inventor	ACHEAMPONG, ANDREW
	Art Unit	1653
	Examiner Name	TBD
	Attorney Docket Number	17618-US-BCON6-AP

Examiner Initial*	Cite No	Foreign Document Number3	Country Code2 j	Kind Code4	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T5
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☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

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☐ See attached certification statement.

☐ Fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ None

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Laura L. Wine/	Date (YYYY-MM-DD)	2013-09-12
Name/Print	Laura L. Wine	Registration Number	68,681

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US	54 72 706 A
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EP	06 96 452 A1

Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

Prüfungsantrag gem. § 44 PatG ist gestellt

(54) Arzneimittel mit einem Gehalt an Ciclosporin

(57) Es wird ein Arzneimittel mit einem Gehalt an Ciclosporin vorgeschlagen, das als Nanoemulsion vorliegt. Außerdem wird die Verwendung eines derartigen Arzneimittels zur Behandlung von Hautkrankheiten und zur Behandlung des menschlichen Auges vorgeschlagen. Ein Verfahren zur Herstellung des Arzneimittels wird ebenfalls bereitgestellt.

DE 198 10 655 A 1

Beschreibung

Die Erfindung betrifft ein Arzneimittel mit einem Gehalt an Ciclosporin.

Derartige Arzneimittel sind allgemein zur Behandlung von Transplantationspatienten bekannt.

Der Wirkstoff Ciclosporin ist ein zyklisches, aus elf Aminosäuren bestehendes Peptid, mit der Summenformel $C_{62}H_{111}N_{11}O_{12}$. Es wird auch als Cyclosporin A (WHO) bezeichnet. Ursprünglich wurde es aus Pilzen isoliert. Inzwischen sind auch Verfahren zu seiner synthetischen Herstellung bekannt.

Ciclosporin ist ein sogenannter Immunmodulator mit immunsuppressiver Wirkung. Es blockiert die Aktivierung von Helfer- und Killerzellen des Immunsystems durch Inhibition der Lymphokinproduktion. Ciclosporin unterdrückt dabei sowohl die humorale als auch die zelluläre Immunreaktion, indem es die Freisetzung von Interleukinen, insbesondere von IL-1 aus Monozyten und IL-2 aus T-Helfer-Zellen in den frühen Phasen der Immunantwort unterbindet.

Aufgrund dieser immunsuppressiven Wirkung wird Ciclosporin zur Vorbeugung der Transplantatabstoßung nach allogenen Transplantationen von Niere, Leber, Herz, Herz-Lunge, Lunge und Pankreas sowie nach Knochenmark-Transplantationen eingesetzt.

Außerdem wird Ciclosporin zur Behandlung der Graft-Versus-Host-Krankheit eingesetzt, einer Krankheit, die bei Transplantationspatienten nach Übertragung fremder immunkompetenter Zellen durch zelluläre Immunreaktionen auftritt.

Weitere Anwendungsgebiete für Ciclosporin sind die Behandlung von schwerer endogener Uveitis, einer schweren Entzündung der Aderhaut des Auges, sowie von schwersten therapieresistenten Formen der Psoriasis (Schuppenflechte). Auch die therapeutische Wirksamkeit von Ciclosporin zur Behandlung des steroidabhängigen und steroidresistenten nephrotischen Syndroms, also von Nierenerkrankungen, die mit einem ausgeprägten Eiweißverlust einhergehen, sind bekannt.

Bisher wurde Ciclosporin als Infusionslösung sowie als Trinklösung von der Sandoz bzw. Novartis AG, Basel dargereicht. Da Ciclosporin ein hydrophobes Peptid ist, das in wäßriger Lösung nicht lösbar ist, enthielten die bisherigen Darreichungsformen als Emulgatoren bzw. Löslichkeitsvermittler in großen Mengen Ethanol (ca. 12 Vol.-%) sowie Lipide in Form von Maiskeimöl und Triacylglycerid-Derivaten.

Die bisher bekannten Verabreichungsformen von Ciclosporin sind lediglich zur systemischen Anwendung geeignet. Wenn Ciclosporin in Form einer Trinklösung verabreicht wird, so erfolgt die Aufnahme in den Körper über den Darm. Bei einer Infusion der Ciclosporin-Lösung gelangt der Wirkstoff direkt ins Blut und verteilt sich über das Blut im gesamten Körper.

Eine topische, d. h. örtlich begrenzte Anwendung von Ciclosporin ist aufgrund seiner lipophilen Eigenschaften, die die Verwendung von Ethanol und Lipiden zum Löslichmachen des Ciclosporins erfordern, problematisch.

Eine andere pharmazeutische Zubereitung, die unter anderem auch für Ciclosporin vorgeschlagen wird, ist in der US 5,154,930 beschrieben. Diese Verabreichungsform umfaßt ein salzfreies geladenes Lipid, wie bspw. Phosphatidylethanolamin oder Phosphatidylserin sowie ein Lösungsmittel wie Polyethylenglykol oder Ethanol. Dabei bilden sich in der pharmazeutischen Zusammensetzung Liposomen-Komplexe zwischen dem Wirkstoff und den Lösungsmitteln. Die beschriebene pharmazeutische Zubereitung erlaubt es, besonders hohe Konzentrationen an Wirkstoff, bspw. Ciclosporin, zu verabreichen.

Als Verabreichungsformen werden Tabletten, Kapseln, Dragees und ähnliches vorgeschlagen. Zur örtlich begrenzten Verwendung an besonders empfindlichen Körperbereichen ist diese Darreichungsform aufgrund der Anwesenheit der zum Löslichmachen der hydrophoben Wirkstoffe notwendigen Lösungsmittel Ethanol bzw. Polyethylenglykol jedoch nicht geeignet.

Die topische Verwendung von hydrophoben Wirkstoffen ist immer dort besonders problematisch, wo stark wasserhaltige bzw. hydrophile Körperteile behandelt werden sollen, da eine Voraussetzung zur Aufnahme solcher Wirkstoffe in den Körper darin besteht, zunächst einen Kontakt zwischen Wirkstoff und Körperoberfläche herzustellen.

Vor diesem Hintergrund ist es die Aufgabe der Erfindung, ein Arzneimittel mit einem Gehalt an Ciclosporin bereitzustellen, das zur topischen Verabreichung auch an stark wasserhaltigen und/oder sehr empfindlichen Körperbereichen geeignet ist, und mit dem eine gute Wirkstoffaufnahme ermöglicht wird.

Es ist eine weitere Aufgabe der Erfindung, neue Anwendungsgebiete für ein derartiges Arzneimittel vorzuschlagen.

Erfindungsgemäß wird diese Aufgabe dadurch gelöst, daß das Ciclosporin in einer Öl-in-Wasser-Nanoemulsion vorliegt. Unter einer Nanoemulsion im Sinne der Erfindung wird jede Öl-in-Wasser-Emulsion verstanden, die Tröpfchengrößen im Nanometerbereich, also mit Durchmessern von kleiner als 1 µm enthält. Derartige Nanoemulsionen haben eine ölige bzw. Lipid-Phase und eine wäßrige Phase, wobei die wäßrige Phase Wasser oder physiologisch verträgliche wäßrige Lösungen wie bspw. physiologische Kochsalzlösung (0,9 Gew.-% Natriumchlorid in Wasser) aufweist.

In einer derartigen Nanoemulsion wird der hydrophobe Wirkstoff Ciclosporin in den winzigen öligen Tröpfchen gelöst, die wiederum in der wässrigen Phase dispergiert sind. Somit ist der Wirkstoff Ciclosporin optimal verteilt. Bei einer Applikation in stark wasserhaltigen Körperbereichen kann so eine besonders gute Verteilung von Ciclosporin und damit eine optimale Wirkstoffaufnahme erreicht werden.

So zeigte sich in einer über sechs Monate andauernden Studie in der Universitätsklinik Tübingen, daß die Verwendung einer erfindungsgemäßen Nanoemulsion sowohl im Bereich des hochempfindlichen Auges als auch im Hautbereich eine gegenüber herkömmlichen Therapieformen wesentlich verbesserte Wirksamkeit und Verträglichkeit aufweist. Dies ist vor allem darauf zurückzuführen, daß aufgrund der neuen Darreichungsform auf die Verwendung physiologisch bedenklicher bzw. unverträglicher Lösungsmittel vollständig verzichtet werden kann, ohne daß dadurch die Wirkung oder Aufnahme von Ciclosporin an den betreffenden Körperteilen beeinträchtigt wird.

Die der Erfindung zugrundeliegende Aufgabe wird somit vollkommen gelöst.

In einer vorteilhaften Ausgestaltung weist die Nanoemulsion Tröpfchengrößen von kleiner als etwa 500 nm auf.

Diese Maßnahme hat den Vorteil, daß der Wirkstoff Ciclosporin besonders gut dispergiert wird und sich damit optimal auf Gewebeoberflächen verteilt und folglich auch besonders gut in das Gewebe aufgenommen wird.

In einer besonders vorteilhaften Ausgestaltung weist das Arzneimittel einen Gehalt an zumindest einem Phospholipid auf.

Unter Phospholipiden versteht man eine Gruppe von Lipiden, die Derivate entweder von Glycerin oder von dem komplexen Alkohol Sphingosin sind. Phospholipide enthalten im Allgemeinen zwei Fettsäuren, die den hydrophoben Bestandteil des Phospholipids bilden, und eine sogenannte

polare Kopfgruppe, die aus einem über eine Phosphodiestergruppe gebundenen Alkohol besteht. Durch diese Struktur sind die Phospholipide amphiphil, d. h. sie enthalten sowohl hydrophobe als auch hydrophile Gruppen. Dadurch sind sie besonders gut als Emulgatoren von hydrophoben Stoffen in wäßrigen Phasen einsetzbar.

Die Verwendung von Phospholipiden in dem erfindungsgemäßen Arzneimittel hat den Vorteil, daß Phospholipide Bestandteile aller Zellmembranen sind und somit eine besonders hohe physiologische Verträglichkeit aufweisen. Dadurch ist das Arzneimittel auch an besonders sensiblen Organen, wie bspw. dem menschlichen Auge, einsetzbar.

In einer weiteren vorteilhaften Ausgestaltung ist das Phospholipid Lecithin.

Lecithin oder Phosphatidylcholin ist eines der am weitesten verbreiteten Membranlipide des Menschen. Von der WHO ist dem Lecithin Unbedenklichkeit als Lebensmittel zuerkannt worden, es wurden keine ADI-Werte (Acceptable Daily Intake) zuerkannt. Lecithin entspricht ferner den Normen der US-amerikanischen Behörde FDA und besitzt den GRAS-Status (Generally Recognized As Safe, CFR Nr. 182.1400/184.1400).

In Fettemulsionen für die parenterale Ernährung wird Lecithin in Kliniken in großem Umfang eingesetzt.

Lecithin ist somit ein besonders gut verträglicher Emulgator, der aufgrund seines Vorkommens in menschlichen Zellen ohnehin Bestandteil des menschlichen Körpers und damit gesundheitlich unbedenklich ist. Als Emulgator von Ciclosporin ist es aufgrund seiner stark amphiphilen Eingenschaften besonders gut geeignet.

In einer weiteren Ausgestaltung der Erfindung liegt der Gehalt an Phospholipid im Bereich von 0,1 bis 20 Gew.-%, vorzugsweise im Bereich von 1 bis 10 Gew.-%.

Hierbei ist vorteilhaft, daß diese Konzentrationen eine besonders feine Emulgierung des Ciclosporins in einer wäßrigen Lösung erlauben.

In einer weiteren Ausgestaltung weist die Nanoemulsion einen Gehalt an Triacylglyceriden, bevorzugt mittelkettigen Triacylglyceriden auf.

Triacylglyceride sind neutrale Lipide, bei denen Fettsäuren über Esterbindungen an einen Glycerinrest gebunden sind. Die Fettsäuren können kurz-, mittel- oder langkettig sein, sie können gesättigt oder ungesättigt vorliegen. Triacylglyceride sind stark hydrophobe Stoffe und dienen z. B. als Energiespeicher im Körper, wo sie in den Fettzellen abgelagert werden.

Die Verwendung von Triacylglyceriden, insbesondere mittelkettigen Triacylglyceriden hat den Vorteil, daß diese problemlos in Arzneimittelqualität erhältlich und zum Inlösungbringen des Ciclosporin in einer Nanoemulsion besonders gut geeignet sind.

Dies gilt vor allem dann, wenn der Gehalt an Triacylglyceriden im Bereich von 10 bis 40 Gew.-%, bevorzugt im Bereich von 20 bis 30 Gew.-% vorliegt.

In einer besonders vorteilhaften Ausgestaltung liegt der Gesamtgehalt an Lipiden im Bereich von 1 bis 50 Gew.-%, bevorzugt im Bereich von 20 bis 30 Gew.-%.

Dabei umfaßt der Gesamtgehalt an Lipiden sowohl rein hydrophoben Lipide wie Triacylglyceride als auch amphiphile Phospholipide wie bspw. Lecithin.

Diese Maßnahme hat den Vorteil, daß bei einem möglichst effizienten Emulgieren des hydrophoben Wirkstoffs Ciclosporin gleichzeitig eine Anwendung an hydrophilen Oberflächen und eine gute Wirkstoffaufnahme möglich ist.

In einer weiteren Ausgestaltung der Erfindung liegt der Gehalt an Ciclosporin im Bereich von 0,1 bis 10 Gew.-%, bevorzugt im Bereich von 1 bis 3 Gew.-%.

Hierbei ist vorteilhaft, daß eine gute therapeutische Wirk-

samkeit von Ciclosporin erzielt wird.

In einer besonders vorteilhaften Ausgestaltung weist das Arzneimittel 2 Gew.-% Ciclosporin, 5 Gew.-% Lecithin, 23 Gew.-% mittelkettige Triacylglyceride und 70 Gew.-% physiologische Kochsalzlösung sowie ggf. ein physiologisch verträgliches Konservierungsmittel auf.

Es hat sich nämlich in einer klinischem Studie mit dem erfindungsgemäßen Arzneimittel herausgestellt, daß mit dieser Zusammensetzung eine besonders gute Verträglichkeit bei optimaler Wirkstoffaufnahme in den Körper erreicht wird.

Der Zusatz eines Konservierungsmittels ist notwendig, wenn das Arzneimittel für längere Zeitspannen aufbewahrt werden soll. Es versteht sich, daß Konservierungsmittel bei allen Verabreichungsformen des erfindungsgemäßen Arzneimittels enthalten sein können.

In einer weiteren Ausgestaltung liegt das erfindungsgemäße Arzneimittel mit physiologisch verträglichen Trägerstoffen zum Auftragen auf die Haut vermischt vor.

Derartige Trägerstoffe können bspw. Cremes, Gele oder Salben mit den üblichen Bestandteilen sein.

Hierbei ist von Vorteil, daß bei der Verwendung der erfindungsgemäßen Nanoemulsion auf der Haut oder einer Schleimhaut die Verteilung sowie das Zurückhalten auf der Haut verbessert wird.

Darüber hinaus wird die Anwendung des Arzneimittels für den Patienten erleichtert.

In einer weiteren Ausgestaltung weist das Arzneimittel viskositätserhöhende Zusätze auf.

Derartige Zusätze können bspw. Zellulosederivate, Polyacrylate oder andere physiologisch verträgliche Polymere sein.

Hierbei ist von Vorteil, daß der Verbleib des Wirkstoffs Ciclosporin an dem Ort, wo er wirken soll, verlängert wird.

Die Erfindung betrifft auch die Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik zur Behandlung von Hautkrankheiten.

Bei in der Hautklinik der Universitätsklinik Tübingen durchgeführten Versuchen wurde nämlich erstmals die topische Anwendung von Ciclosporin zur Behandlung von Hautkrankheiten untersucht. Dabei stellte sich heraus, daß durch die topische Verabreichung von Ciclosporin gegenüber den üblicherweise verwendeten Therapien, insbesondere der Verabreichung von Cortisonpräparaten, eine überlegene therapeutische Wirkung bei gleichzeitiger guter Verträglichkeit erzielt wird.

Da Ciclosporin bisher nur systemisch angewendet wurde, kam die Behandlung von gewöhnlichen lokal begrenzten Hautkrankheiten aufgrund der schweren Nebenwirkungen einer systemischen Anwendung nicht in Betracht. Bei der systemischen Verabreichung kommt es aufgrund der immunsupprimierenden Wirkung des Ciclosporins nämlich zu einer erhöhten Anfälligkeit gegen Infektionen jeglicher Art. Diese Nebenwirkungen wurden bisher nur bei schweren, sonst nicht behandelbaren Krankheiten in Kauf genommen.

Galeniken, die bei einer erfindungsgemäßen Verwendung in Betracht kommen, umfassen z. B. die Formulierung als Cremes, Gele, Salben oder auch in Form von Liposomen oder Mikroemulsionen.

Besonders bevorzugt ist jedoch die Verwendung von Ciclosporin in Form einer Nanoemulsion, wie sie weiter oben beschrieben wurde.

Hierbei ist vorteilhaft, daß sich der Wirkstoff Ciclosporin bei einer Verabreichung als Nanoemulsion in der oberen Hautschicht, der Hornschicht, anreichert. Dadurch wird ein besonders langer Wirkstoffverbleib in diesen Hautbezirken erreicht, was erwünscht ist, da bei den meisten Hautkrank-

heiten die obersten Hautzellschichten befallen sind.

In einer besonders vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel im Bereich der Mundschleimhaut und/oder der Schleimhäute des Genitalbereichs verwendet.

Im Bereich dieser stark wasserhaltigen Oberflächen ist eine rasche Aufnahme des hydrophoben Wirkstoffs zwingend erforderlich, da er an diesen Körperoberflächen nicht anhaftet und vor allem im Mundbereich durch Speichel schnell weggespült wird. Die erfindungsgemäße Nanoemulsion sorgt dabei für die Anlagerung von Ciclosporin an die Schleimhäute und fördert somit eine schnelle Aufnahme.

In einer weiteren vorteilhaften Ausgestaltung wird das Arzneimittel zur Behandlung von Lichen ruber eingesetzt.

Diese Krankheit ist eine sehr verbreitete entzündliche Erkrankung der Haut und Schleimhaut, die auch als kleinpapulöses Exanthem oder Flechte bezeichnet wird.

Zur Behandlung dieser Hautkrankheit wurden bisher lediglich Schälkuren mit Vitamin A-Säure und anschließende Hydrocortisonbehandlung oder Behandlung mit anderen Cortisonpräparaten eingesetzt. Im Genitalbereich waren zur Behandlung von Lichen ruber bisher sogar operative Eingriffe erforderlich, die durch die topische Anwendung von Ciclosporin nun unterbleiben können.

In einer weiteren Ausgestaltung wird das erfindungsgemäße Arzneimittel zur Behandlung von Neurodermitis eingesetzt.

Bei einem Vergleich der Ciclosporin-Anwendung und der bisher üblichen Hydrocortisonanwendung konnten verbesserte Therapieerfolge mit Ciclosporin bei der Behandlung von Neurodermitis beobachtet werden.

In einer weiteren vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel zur Behandlung von Neurodermitis im Bereich des Auges verwendet.

Hierbei ist vorteilhaft, daß Ciclosporin, insbesondere wenn es in Form einer erfindungsgemäßen Nanoemulsion vorliegt, keine Reizungen im Auge oder in den Bereichen um das Auge herum hervorruft, wobei es gleichzeitig gut aufgenommen wird, und daß es hoch effizient gegen Neurodermitiden im Augenbereich wirkt, wie in an der Augenklinik der Universitätsklinik Tübingen durchgeführten Versuchen mit Patienten nachgewiesen werden konnte.

Die Erfindung betrifft auch die Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik zur Behandlung von Allergien.

In einer an der Augenklinik der Universitätsklinik Tübingen durchgeführten Studie zeigte sich nämlich, daß Ciclosporin bei topischer Anwendung therapeutisch hochwirksam gegen Allergien eingesetzt werden kann. Ciclosporin kann dabei in allen zur topischen Verabreichung geeigneten Galeniken eingesetzt werden. Besonders bevorzugt ist dabei eine Darreichung als Nanoemulsion, wie sie oben näher beschrieben wurde.

Insbesondere bei einer Verabreichung von Ciclosporin als Nanoemulsion zur Bekämpfung von Allergien im Augenbereich konnten hervorragende Therapieerfolge erzielt werden.

In einer weiteren vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel zur prophylaktischen und/oder therapeutischen Behandlung des Auges verwendet.

Eine therapeutische Behandlung des menschlichen Auges mit Ciclosporin ist z. B. bei Hornhaut-Transplantationen zur Verhinderung von Abstoßungsreaktionen erforderlich.

Bei der Verwendung von Ciclosporin als Nanoemulsion verteilt sich der hydrophobe Wirkstoff besonders gut über den gesamten Augapfel, so daß aufgrund der großen Resorptionsfläche eine optimale Wirkstoffaufnahme gegeben ist. Die Nanoemulsion verteilt sich außerdem im Kammerwasser selbst, das auch die Linse und die Hornhaut umspült. Da das Kammerwasser nur ca. alle vier Stunden ausgetauscht wird, kann Ciclosporin besonders dauerhaft auf die von dem Kammerwasser benetzten Augenbereiche einwirken. So kann das Risiko von Gewebeabstoßungen im Bereich des Auges sicher vermieden werden.

In klinischen Versuchen, bei denen die Verwendung des erfindungsgemäßen Arzneimittels am Auge getestet wurde, kam es in keinem einzigen Fall zu Sehbeeinträchtigungen oder einer Verstopfung der Schlemm-Kanäle, die dem Abfließen der Tränenflüssigkeit in die Nase dienen. Darüber hinaus wurden keine Schmerzfälle beobachtet.

In einer besonders vorteilhaften Ausgestaltung wird das erfindungsgemäße Arzneimittel zur Verhinderung von Abstoßungsreaktionen nach Transplantationen, vorzugsweise im Bereich des Auges, verwendet.

Hierbei ist bspw. an die bereits erwähnten Hornhaut-Transplantationen oder Transplantationen anderer Bestandteile des Auges, jedoch auch an Haut-Transplantationen zu denken.

Da der Wirkstoff nun direkt am Zielort aufgetragen werden kann und dort auch gut aufgenommen wird, kann das Ciclosporin mit im Vergleich zur systemischen Anwendung geringen Nebenwirkungen effizient therapeutisch wirken.

In einem Verfahren zur Zubereitung des erfindungsgemäßen Arzneimittels werden die folgenden grundsätzlichen Schritte durchgeführt:

a) Lösen von Ciclosporin in einer öligen Phase;
b) Hinzufügen eines Anteils einer wäßrigen Phase;
c) Rühren;
d) Hinzufügen des verbleibenden Anteils der wäßrigen Phase;
e) Behandeln des Gemischs mit Ultraschall; und
f) Sterilfiltrieren.

Hierbei ist von Vorteil, daß eine erfindungsgemäße Nanoemulsion mit Ciclosporin in einem zügigen Verfahren ohne technischen Aufwand hergestellt werden kann.

Die ölige Phase kann dabei z. B. Triacylglyceride und Lecithin, die wäßrige Phase physiologische Kochsalzlösung oder Wasser enthalten. Das Lösen des Ciclosporins in Schritt a) sowie das Rühren in Schritt c) kann z. B. durch auf einem Magnetrührer oder mit einem Flügelrührer durchgeführt werden. Es versteht sich, daß das erfindungsgemäße Verfahren unter sterilen Bedingungen durchgeführt werden muß, wobei zwischen den einzelnen aufgeführten Schritten jeweils Sterilfiltrationsschritte zwischengeschaltet werden können.

Die Ultraschallbehandlung dient der Dispersion der öligen Phase in der wäßrigen Phase, wobei die Tröpfchengröße in der entstehenden Suspension durch die Dauer der Ultraschallbehandlung und die Leistung bestimmt wird.

Es versteht sich, daß die vorstehend genannten und die nachstehend noch zu erläuternden Merkmale nicht nur in den angegebenen Kombinationen, sondern auch in anderen Kombinationen oder in Alleinstellung einsetzbar sind, ohne den Rahmen der vorliegenden Erfindung zu verlassen.

Weitere Merkmale und Vorteile der Erfindung ergeben sich aus den nachfolgenden Ausführungsbeispielen.

Beispiel 1

Herstellung einer Ciclosporin-Nanoemulsion

Es wird eine Ciclosporin-Nanoemulsion hergestellt, die aus 2 Gew.-% Ciclosporin, 23 Gew.-% Oleum neutrale

DAB, 5 Gew.-% Lecithin und 70 Gew.-% 0,9%iger Natriumchloridlösung besteht. Die fertige Emulsion ist u. a. zur Verwendung als Augentropfen geeignet.

Inhaltsstoffe und ihre Bezugsquellen:

Ciclosporin: Firma Synochem, Hamburg;

Oleum neutrale DAB (MIGLYKOL): Firma Henkel, Düsseldorf;

Lecithin (80 Gew.-% Phosphatidylcholin): Firma Lipoid, Ludwigshafen;

Natriumchlorid, 0,9 Gew.-%: Firma Braun, Melsungen.

Alle verwendeten Hilfsmittel, bspw. Bechergläser, Rührer, Filter, usw. werden bei 121°C für 15 Minuten lang durch Autoklavieren sterilisiert.

Zunächst wird der lipophile Wirkstoff Ciclosporin zusammen mit dem Lecithin in Oleum neutrale gelöst.

Dazu werden in ein steriles Becherglas 5 g Lecithin, 2 g Ciclosporin und 23 ml Oleum neutrale eingefüllt und mit einem Magnetrührer in Lösung gebracht.

Die Lösung wird in ein zweites Becherglas sterilfiltriert. Dann werden 40 ml 0,9%ige Natriumchloridlösung zugefügt und für 1 Stunde bei 400 upm (Umdrehungen pro Minute) mit einem Flügelrührer gerührt.

Dadurch wird eine Voremulsion erzeugt, die in eine sterile Durchflußzelle gegeben und mit der restlichen Menge an 0,9%iger Natriumchloridlösung aufgefüllt wird.

Diese Lösung wird für 15 Minuten mit 70 Watt Leistung in einem Ultraschallgenerator (Firma Branson, Schwäbisch Gmünd) beschallt.

Dabei wird eine Nanoemulsion mit Tröpfchengrößen von kleiner als 500 nm erzeugt, die über einen 0,45 µm-Sterilfilter direkt in Augentropfflaschen abgefüllt wird.

Alle Arbeitsschritte werden unter einer sterilen Werkbank (Laminar Airflow Bank, Firma Ehret, Emmendingen) durchgeführt. Die Augentropfen sind bei einer Lagertemperatur von 4°C für drei Monate lang steril.

Beispiel 2

Studie mit Patienten an der Universitätsklinik Tübingen

1. Augenklinik

In der Augenklinik der Universitätsklinik Tübingen wurden die in Beispiel 1 hergestellten Augentropfen für einen Zeitraum von sechs Monaten mit insgesamt über 200 Präparationen bei Patienten mit Hornhaut-Transplantationen eingesetzt. Die Studie dauert noch an.

Über den gesamten Zeitraum der Behandlung von Patienten mit dem erfindungsgemäßen Arzneimittel in Form von Augentropfen wurde kein einziger Fall von Schmerzentwicklung bei der Verabreichung der Tropfen beobachtet.

Obwohl die Nanoemulsion ein milchiges Aussehen aufweist, kam es bei der Applikation am Auge in keinem Fall zu Sehbeeinträchtigungen.

Die erfindungsgemäße Ciclosporin-Nanoemulsion wurde außerdem zur Behandlung von Neurodermitiden im Bereich des menschlichen Auges sowie zur Behandlung von Allergien im Augenbereich eingesetzt. Bei beiden Krankheitsbildern konnten überragende Therapieerfolge erreicht werden, ohne daß es zu einer Entwicklung von Schmerzen oder Sehbeeinträchtigungen bei den Patienten gekommen wäre.

2. Hautklinik

Die Ciclosporin-Nanoemulsion wurde darüber hinaus vier Monate lang in der Hautklinik der Universitätsklinik Tübingen zur Behandlung von Lichen ruber im Gesichtsbereich und im Genitalbereich eingesetzt.

Durch den Einsatz von Ciclosporin bei der Behandlung im Gesichtsbereich konnten die bisher üblichen Schälkuren mit Vitamin A-Säure und anschließender Hydrocortisonbehandlung vermieden werden.

Bei der Verwendung im Genitalbereich war es darüber hinaus möglich, auf die bisher üblichen operativen Eingriffe zu verzichten.

Die erfindungsgemäße Ciclosporin-Nanoemulsion wurde außerdem zur Behandlung von Neurodermitis eingesetzt. Hier konnte ein verbesserter Therapieerfolg im Vergleich zu einer Hydrocortisonbehandlung erreicht werden.

In der Hautklinik wurde das erfindungsgemäße Arzneimittel sowohl stationär als auch ambulant eingesetzt. Auch die Studien in der Hautklinik dauern noch an. Darüber hinaus werden derzeit Versuche zur Verabreichung von Ciclosporin zur Behandlung von Hautkrankheiten in Form von Liposomen durchgeführt.

Patentansprüche

1. Arzneimittel mit einem Gehalt an Ciclosporin, **dadurch gekennzeichnet**, daß das Ciclosprorin in einer Öl-in-Wasser-Nanoemulsion vorliegt.
2. Arzneimittel nach Anspruch 1, dadurch gekennzeichnet, daß die Nanoemulsion Tröpfchengrößen von kleiner als etwa 500 nm aufweist.
3. Arzneimittel nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß es einen Gehalt an zumindest einem Phospholipid aufweist.
4. Arzneimittel nach Anspruch 3, dadurch gekennzeichnet, daß das Phospholipid Lecithin ist.
5. Arzneimittel nach Anspruch 3 oder 4, dadurch gekennzeichnet, daß der Gehalt an Phospholipid im Bereich von 0,1 bis 20 Gew.-%, vorzugsweise im Bereich von 1 bis 10 Gew.-% liegt.
6. Arzneimittel nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß die Nanoemulsion einen Gehalt an Triacylglyceriden, vorzugsweise mittelkettigen Triacylglyceriden aufweist.
7. Arzneimittel nach Anspruch 6, dadurch gekennzeichnet, daß der Gehalt an Triacylglyceriden im Bereich von 10 bis 40 Gew.-%, insbesondere im Bereich von 20 bis 30 Gew.-% liegt.
8. Arzneimittel nach einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, daß der Gesamtgehalt an Lipiden im Bereich von 1 bis 50 Gew.-%, vorzugsweise im Bereich von 20 bis 30 Gew.-% liegt.
9. Arzneimittel nach einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, daß der Gehalt an Ciclosporin im Bereich von 0,1 bis 10 Gew.-%, vorzugsweise im Bereich von 1 bis 3 Gew.-% liegt.
10. Arzneimittel nach einem der Ansprüche 1 bis 9, dadurch gekennzeichnet, daß es 2 Gew.-% Ciclosporin, 5 Gew.-% Lecithin, 23 Gew.-% mittelkettige Triacylglyceride und 70 Gew.-% physiologische Kochsalzlösung sowie ggf. ein physiologisch verträgliches Konservierungsmittel aufweist.
11. Arzneimittel nach einem der Ansprüche 1 bis 10, dadurch gekennzeichnet, daß es mit physiologisch verträglichen Trägerstoffen zum Auftragen auf die Haut vermischt vorliegt.
12. Arzneimittel nach einem der Ansprüche 1 bis 11, dadurch gekennzeichnet, daß es einen Gehalt an zumindest einem viskositätserhöhenden Zusatz aufweist.
13. Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik, vorzugsweise nach einem der Ansprüche 1 bis 12, zur Behandlung von Hautkrankhei-

ten.

14. Verwendung nach Anspruch 13 zur Behandlung der Mundschleimhaut und/oder der Schleimhäute des Genitalbereichs.

15. Verwendung nach einem der Ansprüche 13 oder 14 zur Behandlung von Lichen ruber.

16. Verwendung nach einem der Ansprüche 13 oder 14 zur Behandlung von Neurodermitis.

17. Verwendung nach Anspruch 16 zur Behandlung von Neurodermitis im Bereich des Auges.

18. Verwendung eines Arzneimittels mit einem Gehalt an Ciclosporin in einer zur topischen Anwendung geeigneten Galenik, vorzugsweise nach einem der Ansprüche 1 bis 12, zur Behandlung von Allergien.

19. Verwendung nach Anspruch 18 zur Behandlung von Allergien im Bereich des Auges.

20. Verwendung eines Arzneimittels nach einem der Ansprüche 1 bis 12 zur prophylaktischen und/oder therapeutischen Behandlung des Auges.

21. Verwendung eines Arzneimittels nach einem der Ansprüche 1 bis 12 zur Verhinderung von Abstoßungsreaktionen nach Transplantationen, vorzugsweise im Bereich des Auges.

22. Verfahren zur Zubereitung eines Arzneimittels nach einem der Ansprüche 1 bis 12, gekennzeichnet durch die grundsätzlichen Schritte:

a) Lösen von Ciclosporin in einer öligen Phase;
b) Hinzufügen eines Anteils einer wäßrigen Phase;
c) Rühren;
d) Hinzufügen des verbleibenden Anteils der wäßrigen Phase;
e) Behandeln des Gemischs mit Ultraschall; und
f) Sterilfiltrieren.

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(54) Solubilization reagent for biological test samples.

(57) A solubilization reagent for use in analytical systems for the determination of hydrophobic analytes in a biological test sample, particularly analytical systems employing specific binding proteins for such analytes, such as in fluorescent polarization immunoassays, is disclosed. The solubilization reagent dissociates analytes from various components of a biological test sample, such as cellular material, phospholipids, proteins and the like, at substantially low concentrations of such solubilization reagent while, at the same, minimizing the denaturation of specific binding proteins, such as, for example, antibodies, which may be present in an analytical system. Preferably, such surfactant is alkyl-oxy-(polyethylene-oxy-propylene-oxy-sopropanol) or N-tetradecyl-n,n-demethyl-3-ammonio-1-propane sulfonate, and may further comprise saponin.

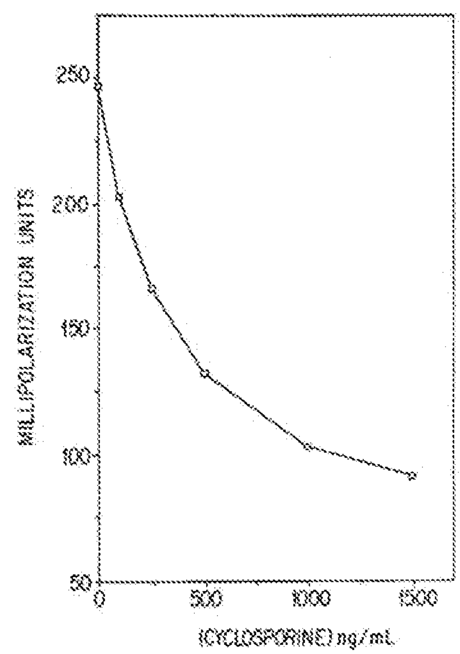

FIG. 1

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Field of the Invention

The present invention relates to reagents which are useful for extracting analytes from a liquid test sample. In particular, the present invention relates to reagents which facilitate the dissociation of analytes, particularly hydrophobic analytes, from the components of a biological test sample to permit the measurement of such analytes present therein.

Background of the Invention

The monitoring of therapeutic drug levels and other analytes in biological fluids such as serum, plasma, whole blood, urine and the like has become very useful to provide physicians with information to aid in proper patient management. For example, adjustment of patient dosage, achievement of optimal therapeutic effects, and avoiding useless subtherapeutic or harmful toxic dosage levels can be provided. Conventional techniques which are employed to monitor drug levels or detect other analytes are known and include radioimmunoassays and nonisotopic assays such as fluorescence polarization immunoassays. However, such techniques produce inconsistencies in results when determining the amount or presence of hydrophobic analytes because of their intracellular relationship with various cellular components of a biological test sample. Accordingly, when such analytes remain associated with such cellular components, the detection of such analytes in an analytical system is difficult, and in some instances impossible, particularly when such analytes are present at particularly low levels.

Although various reagents have been described to extract various analytes for analysis, such as Triton X-100[R], Tweens[R], sodium dodecyl sulfate and saponin, the use of such reagents suffer from a number of disadvantages, particularly where such analysis involves reagents such as specific binding proteins, antibodies, and the like. For example, such reagents, in many cases, do not achieve complete cell lysis wherein in the case of hydrophobic analytes, a significant amount of such analytes could remain associated with cellular components and thereby not made available for analysis. Similarly, the presence of such reagents in, for example, an immunoassay system, will result in significant denaturation of specific binding proteins or antibodies employed in such immunoassays to thereby reduce the binding activity of such proteins and antibodies. Moreover, the use of such reagents to dissociate analytes from various cellular components and other materials which may be present in a liquid test sample can have a dramatic effect on the integrity of reagents employed in various analytical systems, particularly where such reagent are employed at high concentrations in order to achieve such dissociation.

Summary of the Invention

The present invention relates to the discovery that analytical systems for the determination of hydrophobic analytes in a biological test sample, particularly analytical systems employing specific binding proteins for such analytes, can be substantially improved by employing the solubilization reagent of the present invention which serves to dissociate analytes from various components of a biological test sample, such as cellular material, phospholipids, proteins and the like. In particular, such solubilization reagent has unexpectedly and surprisingly been found to dissociate hydrophobic analytes from such components, particularly cellular components, at substantially low concentrations of such solubilization reagent while, at the same, minimize the denaturation of specific binding proteins, such as, for example, antibodies, which may be present in an analytical system. The solubilization reagent of the present invention is particularly useful in a fluorescent polarization immunoassay for the determination of hydrophobic analytes such as cyclosporine and the like.

The solubilization reagent of the present invention comprises from between about 1.5% (w/v) and about 10% (w/v), preferably about 2% (w/v), of a surfactant having either nonionic characteristics or zwitterionic characteristics wherein such surfactant is capable of dissociating substantially all of a hydrophobic analyte from the components of a biological test sample. Preferably, such surfactant is either a nonionic polyglycol detergent, such as alkyl-oxy(polyethylene-oxy-propylene-oxy-isopropanol), or a zwitterionic detergent, such as N-tetradecyl-N,N-demethyl-3-ammonio-1-propanesulfonate. The solubilization reagent may further comprise from between about 0% (w/v) and about 25% (w/v) of saponin.

Brief Description of the Drawings

Fig. 1 illustrates a calibration curve employed to determine the amount cyclosporine from a whole blood sample in a fluorescent polarization immunoassay employing the solubilization reagent of the present invention.

Detailed Description of the Invention

The solubilization reagent of the present invention dissociates analytes, particularly hydrophobic analytes, from a biological test sample such as whole blood, serum, plasma, urine, spinal fluid, and the like. As contemplated by the present invention,

hydrophobic analytes include, but are not intended to be limited to, steroids, drugs such as cyclosporine, and the like.

In particular, the solubilization reagent dissociates such analytes from cellular material, such as erythrocytes, populations of leucocytes, such as lymphpocytes, phospholipids, proteins, and the like, which may be present in a biological test sample, to thereby render such analytes readily available for measurement by a desired analytical system. Although the solubilization reagent is particularly useful in analytical systems for determining hydrophobic analytes employing specific binding proteins, especially immunoassay systems, the solubilization reagent can be be employed in other assay systems as well, such as radioactive assays and the like.

Where it is desireable to employ a non-ionic surfactant in the solubilization reagent according to the present invention, such non-ionic surfactant is preferably a nonionic polyglycol detergent such as alkyloxy(polyethyleneoxypropyleneoxy)-isopropanol and the like, also commonly known as Tergitol®. Where it desireable to employ a zwitterionic surfactant in the solubilization reagent according to the present invention, such zwitterionic surfactant is selected from the group consisting of N-tetradecyl-N,N-demethyl-3-ammonio-1-propanesulfonate, N-octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-hexadecyl-N,N-dimethyl_3-ammonio-1-propanesulfonate, and the like.

In addition to either a non-ionic surfactant or a zwitterionic surfactant as described above, the solubilization reagent according to the present invention may further comprise a glycoside, such as saponin.

According to the present invention, the solubilization reagent is capable of lysing substantially all of the various cellular components which may be present in a biological test sample, and dissociate substantially all of the desired analyte from other biological test sample components in order to render such analyte available for analysis. In particular, the solubilization reagent according to the present invention is capable of providing substantially complete cell lysis of, for example, cellular populations such as erythrocytes, leukocytes and the like, for recovery of substantially all of the desired analyte contained therein. In addition, the solubilization reagent is also capable of dissociating the desired analyte from other components which may be present in a biological test sample, such as cellular material, phospholipids, proteins, and the like, to which such analyte could nevertheless remain associated with and thereby not made available for analysis.

The solubilization reagent according to the present invention has unexpectedly and surprisingly been found to achieve such cell lysis and dissociation of analyte at substantially low concentration. In particular, the use of the solubilization reagent at a concentration as low as 1.5% (w/v) of the non-ionic or zwitterionic surfactant, with saponin, has been found to be effective for such cell lysis and dissociation of the analyte. Preferably, the concentration of the surfactant is from between about 1.5% (w/v) and about 10% (w/v), more preferably about 2% (w/v), and, the solubilization reagent may further comprise from between about 0% and about 25% saponin, preferably 2%.

It is to be understood that the use of reagents to treat a biological test sample prior to the use thereof in an analytical system, such as described herein, will be present in the biological test sample during subsequent analysis thereof. Accordingly, the solubilization reagent of the present invention is particularly useful where the biological test sample is to be employed in an analytical system employing specific binding proteins or antibodies which are sensitive to the presence of, for example, detergents or other pretreatment reagents which are typically employed for the purposes described herein. For example, the use of the solubilization reagent according to the present invention prior to the analysis thereof in an immunoassay system minimizes denaturation of antibody reagents employed therein, thereby having substantially no effect on the binding activity of such antibody reagents.

When employing the solubilization reagent of the present invention for performing an immunoassay, the test sample is first treated with the solubilization reagent wherein cellular populations present in the test sample are lysed and the hydrophobic analyte dissociated from other components as described above. The resulting solution is then treated with a precipitation reagent, such as described in the copending U.S. Patent Application Serial No. 567,853 , entitled "Protein Precipitation Reagent", filed on August 15, 1990 and incorporated by reference herein. Such precipitation reagent precipitates any interfering proteins, including the cellular material resulting from treatment of the test sample with the solubilization reagent of the present invention. Although the precipitated material resulting from such pretreatment step with the precipitation reagent may settle by gravity, extraction of the resulting dissociated analyte is preferably accomplished by centrifuging the treated test sample wherein the resulting supernatant contains the desired analyte, substantially free of such cellular material and components. The supernatant is then combined with a detectable tracer com-

pound as would be known by one skilled in the art, and an appropriate antibody to, or binding agent for, the analyte prepared according to methods known in the art. According to such general immunoassay procedure, the analyte present in the test sample and the tracer compound compete for a limited number of binding sites, resulting in the formation of analyte and tracer compound complexes. By maintaining a constant concentration of the tracer compound and the antibody, the ratio of the formation of analyte complex to tracer complex is directly proportional to the amount of analyte present in the test sample.

The solubilization reagent of the present invention is particularly useful in fluorescence polarization immunoassay systems wherein the amount of analyte in a test sample is determined by exciting an assay mixture with polarized light and measuring the polarization of the fluorescence emitted by any of the free or unbound tracer compound and tracer-antibody complex. Any of the tracer compound which is not complexed to an antibody is free to rotate in less than the time required for adsorption and re-emission of fluorescent light. As a result, the re-emitted light is relatively randomly oriented so that the fluorescence polarization of any of the tracer compound not complexed to the antibody is low, approaching zero. Upon complexing with a specific antibody, the tracer-antibody complex thus formed assumes the rotation of the antibody molecule, which is slower than that of the relatively small tracer compound molecule, thereby increasing the polarization observed. When making such determination, the analyte competes with the tracer compound for antibody sites wherein the observed polarization of fluorescence of the tracer-antibody complex becomes a value between the value of the free tracer compound and the value tracer-antibody complex. Accordingly, if the test sample contains a high concentration of analyte, the observed polarization value is closer to that of the free tracer compound, i.e., low. Conversely, if the test sample contains a low concentration of analyte, the polarization value is closer to that of the tracer-antibody complex, i.e., high. By sequentially exciting the reaction mixture of an immunoassay with vertically and then horizontally polarized light, and analyzing only the vertical component of the emitted light, the polarization of the fluorescence in the reaction mixture can be accurately determined. The precise relationship between polarization and concentration of the analyte is established by measuring the polarization values of calibrators having known concentrations, and the concentration of the analyte can be interpolated from a standard curve prepared therefrom.

When employing fluorescence polarization techniques, the results can be quantified in terms of "millipolarization units", "span" (in millipolarization units) and "relative intensity". The measurement of millipolarization units indicates the maximum polarization when a maximum amount of the tracer compound is bound to the antibody in the absence of any phenylclorobenzene (PCB) in the test sample. The higher the net millipolarization units, the better the binding of the tracer compound to the antibody. For the purposes of the present invention, a net millipolarization value of at least about 130 is preferred.

The "span" is an indication of the difference between the net millipolarization and the minimum amount of the tracer compound bound to the antibody. A larger span provides for a better numerical analysis of the data. For the purposes of the present invention, a span of at least about 15 millipolarization units is preferred.

The "relative intensity " is a measure of the strength of the fluorescence signal above the backgound fluorescence. Thus, a higher intensity will give a more accurate measurement. The intensity is determined as the sum of the vertically polarized intensity plus twice the horizontally polarized intensity. The intensity can range from a signal of about three times to about thirty times the backgound noise, depending upon the concentration of the tracer compound and other assay variables. For the purpose of the present invention, an intensity of about three to about twenty times that of background noise is preferred.

The solubilization reagent according to the present invention is particularly useful for performing a fluorescent polarization immunoassay for cyclosporine and metabolites thereof employing a fluorescent tracer compound comprising 4-aminomethylfluorescein coupled to the hydroxyl group of MeBmt at the first position of cyclosporine, as described by the copending U.S. Patent Application Serial No. 567,842 , entitled "Immunoassay Reagents And Method For Determining Cyclosporine", filed on even date herewith and incorporated by reference herein, and a monoclonal antibody to cyclosporine, such as described by International Patent Application Publication No. WO 86/02080. According to such method, a precipitation reagent comprising zinc sulfate, ethylene glycol and methanol, such as described in the copending U.S. Patent Application Serial No. 567,853 , entitled "Protein Precipitation Reagent", filed on August 15, 1990 and incorporated by reference herein, a dilution buffer, and calibrators and controls are also employed. Such precipitation reagent is employed to precipitate interfering proteins, hemoglobin, and other interfering substances while, at the same time, maintaining hydrophobic analytes in solution in order to render such analytes available for binding to, for example, a spe-

cific binding protein such as an antibody.

Once the the test sample has been treated with the solubilization reagent of the present invention and the precipitation reagent as described above, the supernatant containing cyclosporine, or cyclosporine and metabolites of cyclosporine, is then combined with the antibody. Prior to addition of the tracer compound and dilution buffer, a background fluorescence reading is taken, wherein after an incubation period of from between about ten minutes and about thirty minutes, a fluorescence polarization reading is taken as described above.

The present invention will now be illustrated, but is not intended to be limited, by the following example:

Fluorescent Polarization Immunoassay For Cyclosporine

Reagents

The reagents for performing a fluorescence polarization immunoassay employing the solubilization reagent according to the present invention were prepared as follows:

a) Cyclosporine Tracer Reagent:

(i) Preparation of [O-(Chloroformyl)MeBmt]¹ cyclosporine (Cyclosporine chloroformate):

Cyclosporine (24.2 mg, 0.020 mmoles) was dissolved in a 25%w/w solution of phosgene in benzene (2.0 mL) in a 10mL round bottom flask fitted with stopper and stirbar. The reaction was stirred for 5 minutes to dissolve the cyclosporine, then was allowed to stand undisturbed at room temperature for 24 hours. The reaction was concentrated in vacuo, and the product could be stored as a solid at 0° C for up to six months. For subsequent reactions, a 0.02M solution in DMF was used.

(ii) Preparation of [O-(Fluorescein-4'-yl-methylaminoformyl)MeBmt]¹ cyclosporine:

Cyclosporine chloroformate (0.2mL, 4 moles), as a 0.02M solution in DMF as described in step (i) above was combined with 4'-aminomethylfluorescein hydrochloride (2.0 mg, 5 moles) in a stoppered vial fitted with a stirbar. Pyridine was added until the apparent pH (by moist pH paper) was approximately 7. The reaction was stirred at room temperature for 24 hours. The solvent was removed in vacuo, and the residue was taken up in methanol and loaded onto a 1mm silica gel plate. The plate was developed with 15% methanol/methylene chloride. The product band, Rf 0.55, was eluted from the silica gel with methanol.

(iii) Preparation of Tracer Reagent:

A 60 nanomolar cyclosporine tracer reagent was prepared comprising the cyclosporine tracer compound prepared according to step (ii) above in 0.1 M sodium phosphate buffer, pH 7.5, containing 0.01 % (w/v) bovine gamma globulin, 0.1 % (w/v) soldium azide, 5.0% (w/v) ethylene glycol and 0.05% (w/v) Tween™ 20.

(b) Monoclonal Antibody Formulation:

A monoclonal antibody reagent was prepared comprising mouse (ascites) monoclonal antibody to cyclosporine (Sandoz AG, Basle, Switzerland) diluted with a citrate buffer including sodium azide.

(c) Pretreatment Reagent:

A pretreatment reagent was prepared comprising 0.1 M Tris™ buffer, pH 7.5, 0.1% (w/v) sodium azide, 0.5% (w/v) copper sulfate and 10.0% (w/v) 5-sulfosalicylate.

(d) Dilution Buffer:

A dilution buffer was prepared comprising 0.1 M sodium phosphate, pH 7.5, and 0.1 % (w/v) bovine gamma globulin.

(e) Whole Blood Precipitation Reagent:

A whole blood precipitation reagent was prepared comprising 60 mM zinc sulfate, 50% (w/v) methanol and 33% (w/v) ethylene glycol.

(f) Solubilization Reagent:

A solubilization reagent was prepared comprising 2.0% (w/v) Tergitol min foam 1X ™, 2.0% (w/v) saponin and 0. 1% (w/v) sodium azide.

(g) Calibrators:

Cyclosporine monoclonal whole blood calibrators were prepared comprising cyclosporine and an artificial human whole blood matrix. The calibrators were prepared at concentrations of 0.0, 100, 250, 500, 1000, and 1500 nanograms per milliliter, with sodium azide as a preservative.

(h) Controls:

Cyclosporine monoclonal whole blood controls were prepared comprising cyclosporine and an artificial whole blood matrix. The controls were prepared at concentrations of 150, 400 and 800 nanograms per milliliter with sodium azide as a preservative.

Cyclosporine Whole Blood FPIA Assay Protocol

A fluorescent polarization immunoassay for determining cyclosporine in a whole blood test sample employing an Abbott TDx® Therapeutic Drug Monitoring Analyzer was performed as follows:

One hundred-fifty microliters each of patient whole blood samples containing cyclosporine, controls and calibrators were pipetted into labeled centrifuge tubes, and 50 microliters of the solubilization

reagent were added to each of the tubes. A pipette was filled with the whole blood precipitation reagent, purged of air bubbles, and 300 microliters were dispensed into each centrifuge tube by touching the end of the pipette tip to the wall of each centrifuge tube while dispensing the reagent. The centrifuge tubes were then capped and mixed on a vortex mixer for ten seconds and placed into a centrifuge head so that the tubes were evenly distributed so that the centrifuge head was balanced. The tubes were centrifuged for approximately five minutes at 9,500 x g until a clear supernatant and a hard, compact pellet of denatured protein was obtained. After centrifugation was complete, each tube was uncapped and the supernatant was decanted into the corresponding sample well of a TDx Sample Cartridge.

The fluorescence polarization value of each calibrator, control and sample was determined and printed on the output tape of the Abbott TDx Analyzer. A standard curve was generated in the instrument by plotting the polarization, P, of each calibrator versus its concentration using a nonlinear regression analysis wherein, the concentration of each control and sample was read off the stored calibration curve (Figure 1) and printed on the output tape.

The sensitivity of the preferred fluorescence polarization assay according to the present invention is 15.0 nanograms/milliliter of cyclosporine and metabolites. When compared to an available radioimmunoassay using 60 clinical samples, a linear least squared regression analysis gave a slope of 0.947, an intercept of 7.15, and a correlation coefficient of 0.969.

Where a test kit according to the present invention is being used in conjunction with the TDx Analyzer, the reagents for performing the fluorescent polarization immunoassay according to the present invention can be contained in separate vials of a TDx Reagent Pack wherein vial caps from each of the vials in the Reagent Pack are removed and placed into designated wells inside the Reagent Pack. Accordingly, once the Reagent Pack is placed inside the TDx Analyzer, the assay procedure heretofore is fully automated.

If a manual assay is being performed, the test sample is first treated with the precipitation reagent as described above, and then mixed with the dilution buffer. The antibody reagent and the pretreatment solution are then placed into the test tube containing the sample, and a backgound fluorescence reading is taken. The tracer compound and dilution buffer are added to the sample, and after incubation, a fluorescence polarization reading is taken.

It will be apparent that many modifications and variations of the present invention as herein set forth are possible without departing from the spirit and scope hereof, and that, accordingly, such limitations are imposed only as indicated by the appended claims.

Claims

1. A solubilization reagent useful for dissociating hydrophobic analytes from components of a biological test sample, said reagent comprising a non-ionic or a zwitterionic surfactant.

2. The reagent of claim 1 wherein said non-ionic surfactant is a non-ionic polyglycol surfactant.

3. The reagent of claim 1 wherein said zwitterionic surfactant is selected from the group consisting of N-tetradecyl-N,N-demethyl-3-ammonio-1-propanesulfonate, N-octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate and N-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate.

4. The reagent of claims 1-3 further comprising a glycoside.

5. The reagent of claims 1-4 comprising from between about 1.5% (w/v) and about 10% (w/v) of said surfactant in aqueous solution.

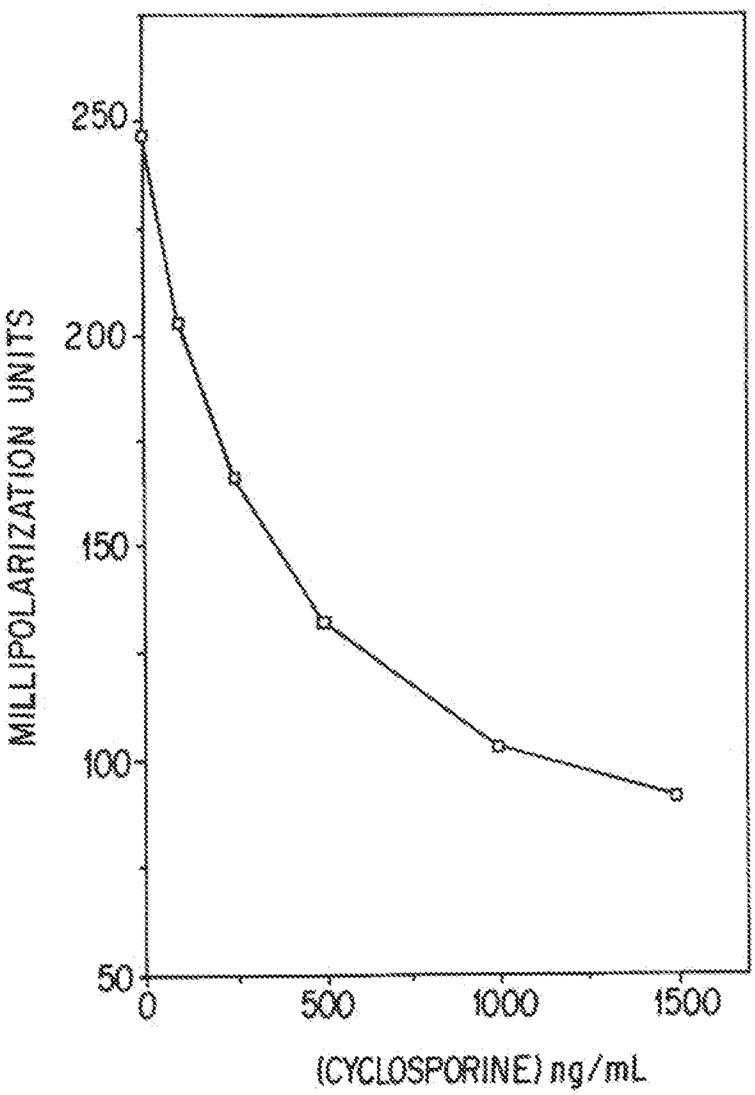
6. The reagent of claim 4 comprising less than about 25% (w/v) of said glycoside.

7. An immunoassay method for determining hydrophobic analytes in a biological test sample characterized in that said assay comprises a solubilization reagent according to claims 1-3.

8. The immunoassay method of claim 7 wherein said reagent further comprises a glycoside.

9. The immunoassay method of claim 7 wherein said reagent comprises from between about 1.5% (w/v) and about 10% (w/v) of said surfactant in aqueous solution.

10. The immunoassay method of claims 7-9 wherein said reagent comprises less than about 25% (w/v) of said glycoside.



250
200
150
100
50
MILLIPOLARIZATION UNITS
0
500
1000
1500
(CYCLOSPORINE) ng/mL

FIG. 1

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(54) **EXTERNAL PREPARATION CONTAINING CYCLOSPORIN.**

(57) An external preparation containing cyclosporin as the active ingredient, characterized by comprising (a) cyclosporin, (b) an organic solvent for dissolving the same, (c) a fatty acid ester of a monohydric alcohol, which is liquid at 25 °C and bears at least 8 carbon atoms in total, and/or an alkanolamine which is liquid at 25 °C, (d) an oleaginous substance which is solid at 25 °C, and (e) a surfactant, wherein the cyclosporin content ranges from 0.1 to 10 wt.% and the content of the ester and/or alkanolamine ranges from 1 to 15 wt.%. The preparation has an excellent efficacy of curing atopic dermatitis, psoriasis, allergic contact dermatitis, and so forth.

EP 0 547 229 A1

TECHNICAL FIELD

The present invention relates to topical preparations containing cyclosporin as a major active component. The topical preparations containing cyclosporin include topical preparations in the form of an emulsion or a non-emulsion.

The term "cyclosporin" referred to in this application is intended to mean a single substance or a mixture of a group of cyclosporin antibiotics which are described in detail in Japanese Patent Laid-open Publication (kokai) No. 2-17,127.

BACKGROUND ART

Cyclosporin is known as an immune inhibitor and it has extensively been employed in the field of the transplant of organs including the kidney. Recently, cyclosporin becomes apparent as being effective to various diseases that are caused mainly from autoimmune reaction, in addition to the efficacy for the transplant of the organs. A number of literature has already been published which reports the efficacy of cyclosporin forarthritis. Autoimmune diseases to which cyclosporin has been applied or proposed to be applied may include, for example, autoimmune blood diseases, chronic bronchial asthma, systemic erythematosus, polymyositis, systemic scleroderma, Wegner syndrome, myasthenia gravis, psoriasis vulgaris, autoimmune intestinal diseases (idiopathic ulcerative colitis, Crohn disease), sarcoidosis, multiple sclerosis, juvenile diabetes mellitus, uveitis, psoriatic rheumatoid, glomerulonephritis, and the like.

As described hereinabove, cyclosporin contributes largely to the inhibition of rejection at the time of transplanting organs and autoimmune therapy; however, it is also known that it may often cause severely adverse affect upon the kidney when administered orally over a long period of time so that this toxicity to the kidney has been the cause of suppressing cyclosporin from being extensively employed. It can be noted that there are many cases where morbid states are caused to occur at the skin, eye or joint to which topical preparations can be applied. In the case of diseases that can be administered with topical preparations, it is advantageous to avoid systemic administration that might cause disturbances to occur in the kidney. If the focus of a disease is restricted to a layer of the dermis, topical administration through the epidermis is more advantageous than other ways of administration because it can save the amount of a medicine to be administered and further the efficacy of the medicine can be enhanced in association with a local rise in the concentration of the medicine, while systemic side effects can be reduced. The way of administration in the form of topical preparations can be said to be one of the most effective drug delivery systems (DDS) for cyclosporin.

On the other hand, it is extremely difficult to formulate cyclosporin into topical preparations so as to maintain its highly therapeutical effect, unlike water-soluble or low-molecular weight, pharmaceutically effective substances. One of the reasons for this difficulty is because the cyclosporin is a large cyclopolypeptide having a molecular weight of larger than 1,200 so that it suffers from the difficulty in allowing cyclosporin to infuse or penetrate through the horny skin layer into the focal site present in the dermis layer. Another reason for the difficulty is because the cyclosporin is insoluble in water and there is the restriction upon the kind of organic solvents in which the cyclosporin can be dissolved. As such specific organic solvents, a lower alkanol such as ethanol or isopropanol may be generally employed. However, such a lower alkanol is too highly irritative to the skin when it is employed for topical preparations in a relatively high concentration, so that safe topical preparations cannot be provided. On the other hand, when the lower alcohol is employed in a relatively low concentration for topical preparations, the ability of the cyclosporin to be dispersed uniformly in the topical preparations may be impaired, thereby providing no topical preparations with a highly therapeutical effect.

Reports on clinical research of cyclosporin ointments have been published to the effect that a 10% cyclosporin formulation may be pharmaceutically effective or ineffective, so that its pharmaceutical effects may or may not be reproduced. Some reports describe specific compositions of cyclosporin formulations yet no clear pharmaceutical effects therefor are described.

For example, Japanese Patent Laid-open Publication No. 2-17,127 discloses compositions which contain, as essential components, cyclosporin and a mono- or polyunsaturated fatty acid or an unsaturated alcohol, each having from 12 to 24 carbon atoms. The mono- and polyunsaturated fatty acids may include, for example, vaccenic acid, linoleic acid, linolenic acid, elaidic acid, erucic acid, and the like. The unsaturated alcohol may include, for example, vaccenyl alcohol, linoleyl alcohol, linolenyl alcohol, elaidyl alcohol, erucyl alcohol, and the like. Further, it describes the compositions are effective to various skin diseases; however, that publication does not specify its pharmaceutical effects and refers merely to the ability of the cyclosporin to infuse or penetrate through the skin and to the concentration of the cyclosporin.

The publication is thoroughly silent about the extent, for example, to which the cyclosporin is effective against psoriatic diseases.

Several cases of skin diseases are reported; many of the literature states that cyclosporin is effective against the skin diseases.

For example, atopic dermatitis is reported in Acta. Derm. Venerol.: Suppl. 144, 136 - 138 (1989) where an alcoholic oily gel of containing cyclosporin at the rate of 10% by weight is effective against atopic dermatitis. Further, Arch. Derm.: 125, p. 570 (1989) reports that an alcoholic oily gel of a 10% (by weight) cyclosporin is effective.

There are reports of contact-type dermatitis, for example, in Arch. Dermato-1: 125, 568 (1989) which reports to the effect that cyclosporin is employed for a human DNCB test with no effect. Further, Contact Dermatitis: 19, 129-132 (1988) makes a review on three formulations: a 10% cyclosporin formulation in Labrafil (polyoxyl-5-oleate, olive oil and ethanol), a 5% cyclosporin formulation in castor oil, and a 5% cyclosporin formulation in castor oil containing 20% propylene glycol; however, it states the results of this review are not so satisfactory that a more effective solvent is required. In addition, Contact Dermatitis, 20, 155-156 states that none of three formulations, or 0.1%, 1% and 10% cyclosporin formulations, are effective at all against contact dermatitis.

Pharmaceutical effect of cyclosporin upon psoriasis is described, for example, in Clin. Res., 34, 1007A (1986), in which it is described that topical administration of cyclosporin is not effective for the therapy to psoriasis, although neither the concentration of cyclosporin nor the composition thereof are specified. It is also described in Lancet 1, 806 (1987) that a 2% by weight cyclosporin (on ointment base) is as effective upon psoriasis as placebo. Further, J. Amer. Acad. Dermatol., 18, 378-379 (1988) describes that a 5% cyclosporin solution in olive oil is equal to the sole use of olive oil that is employed as the base in the previous case. In addition, J. Amer. Acad. Dermatol., 22, 126-127 (1990) states that a gel comprising 10% cyclosporin, 43% olive oil, 10% ethanol, 30% polyoxyl-5-oleate and 7% colloidal silica did not produce any effect upon psoriasis. Furthermore, it is reported in Brit. J. Derm., 122, 113-114 (1990) that a 5% (by weight) cyclosporin ointment was not effective.

Reports on alopecia areata are made, for example, in Lancet, 2, 803-804 (1986) where it is described that a 2% cyclosporin oily solution was effective. In addition, Lancet 2, 971-972 (1986) reports that a 5% (wc) cyclosporin formulation in oil was effective against alopecia areata. On the other hand, Acta. Derm. Venereol., 69, 252-253 (1989) describes that a 10% cyclosporin oily preparation was not effective. Furthermore, J. Amer. Acad. Dermatol 22, 251-253 (1989) reports that a 5% cyclosporin formulation was effective against male alopecia, although no specific compositions are described therein.

As long as literature as described hereinabove has been reviewed, it is considerably difficult to draw a conclusion that cyclosporin is topically effective against the skin diseases as specified hereinabove. Even if it could be said that cyclosporin would be effective against the skin diseases, it can be said that cyclosporin should be employed in a considerably large amount. If cyclosporin preparations are not topically effective against the skin diseases or the effect is not satisfactory, it can be said in many occasions that the kinds of formulation components and the dosage are inappropriate. In summary, no conventional topical cyclosporin preparations can achieve the object to utilize cyclosporin effectively as topical preparations.

DISCLOSURE OF INVENTION

The primary object of the present invention is to provide a topical preparation containing cyclosporin, which acts effectively upon skin diseases, is useful therefor, and is highly safe.

Another object of the present invention is to provide a topical preparation containing cyclosporin, which is lower in the concentration of a lower alcohol and high in safety.

A further object of the present invention is to provide a highly safe topical preparation containing cyclosporin, which does not yet contain any quantity of a lower alcohol.

As a result of extensive research and reviews on cyclosporin-containing topical preparations which are superior in the ability of infusion or penetration through the skin or the horny skin layer yet which are less in irritation to the skin and high in safety, the present invention has been completed on the basis of the new finding as will be described hereinafter.

One aspect of the present invention provides the topical preparation containing cyclosporin, which is characterized by (a) cyclosporin; (b) an organic solvent in which the cyclosporin is to be dissolved; (c) an ester of an fatty acid with a monovalent alcohol, which is in liquid state at 25° C and which has a total number of carbon atoms of 8 or more, and/or an alkanol amine in liquid form at 25° C; (d) an oily substance in a solid form at 25° C; and (e) a surfactant, wherein an amount of the cyclosporin ranges from 0.1% by weight to 10% by weight and a total amount of the ester of the fatty acid with the monovalent

alcohol and/or the alkanol amine ranges from 1% by weight to 15% by weight.

Another aspect of the present invention provides a topical preparation containing cyclosporin, which is characterized by (a) cyclosporin; (b) a lower alcohol; (c) an fatty acid ester in liquid state at 25° C and/or an alkanol amine in liquid state at 25° C; (d) an oily substance in solid state at 25° C; and (e) a surfactant, wherein an amount of the cyclosporin ranges from 0.1% by weight to 10% by weight, an amount of the lower alcohol ranges from 2% by weight to 15% by weight; and a total amount of the fatty acid ester and/or the alkanol amine ranges from 1% by weight to 15% by weight.

The cyclosporin-containing topical preparations according to the present invention are characterized by the features that the compositions are different from those of the conventional cyclosporin topical preparations as reported in the aforesaid literature and it can achieve the objects of the present invention in an effective way by using a reduced amount of cyclosporin.

The topical preparations containing cyclosporin according to the present invention is provided with the features as follows:

1. They are superior in therapeutic effect;
2. They are highly stable (i.e., cyclosporin does not become free from the topical preparations, no crystallization of cyclosporin is caused to occur, and no chemical reaction of cyclosporin is caused to occur with any other components of the compositions);
3. They are easily administered topically;
4. They contain cyclosporin in a highly uniformly dispersed state; and
5. They are highly safe.

In order to determine the formulations of the topical preparations according to the present invention, the selection of each component of the formulation and the rates of the components are of significant factors. For example, when the topical preparations are employed in the form of ointment, the pharmaceutical effect of the ointment, the biological activity of the ointment, and the physicochemical stability of the ointment should be taken into account. Heretofore, in usual cases, a higher saturated fatty acid or an fatty acid such as oleic acid or 12-hydroxystearic acid has been employed as an ointment base. Among those fatty acids, lauric acid, myristic acid, palmitic acid and stearic acid have been employed to form soap, together with an alkali, particularly potassium hydroxide, which in turn helps emulsify the formulated medicine.

It should be noted herein, however, that the fatty acid, whether it is employed as it is or in the form of potassium soap as an ointment base, for the cyclosporin-containing topical preparations according to the present invention, is little effective for emulsifying cyclosporin in the topical preparations, whereby no topical preparations with an highly pharmaceutical effect can be provided, and the stability of ointment may be impaired.

BEST MODES FOR CARRYING OUT THE INVENTION

The topical preparations according to the present invention contains cyclosporin, as a major active component, at a rate ranging from 0.1% to 10% by weight, preferably from 1% by weight to 7% by weight. It is to be noted herein that the topical preparations of the present invention can demonstrate highly therapeutic effects in such a low concentration.

The topical preparations according to the present invention contains the organic solvent for cyclosporin, which is in liquid state at ambient temperature (25° C) and which can dissolve the cyclosporin. Such organic solvents may include an aliphatic alcohol and a fatty acid ester with a polyvalent alcohol.

As the aliphatic alcohols, there may be employed any lower alcohol and higher alcohol as long as they are liquid at ambient temperature. The alcohol may be a straight or branched one or may be saturated or unsaturated one. Specific examples of such aliphatic alcohols may include a lower alcohol such as ethanol, propanol, isopropanol, butanol, and the like, and a higher alcohol such as octyl alcohol, nonyl alcohol, decyl alcohol, 2-octyl dodecanol, 2,6-dimethyl-4-heptanol, oleyl alcohol, and the like. The branched higher alcohol is preferably appropriate as the organic solvent for the cyclosporin.

The polyvalent alcohol-fatty acid ester may be represented by the following formula:

R^1COOR^2OH

where

R^1 is an alkyl group having from 4 to 12 carbon atoms, preferably from 6 to 10 carbon atoms; and
R^2 is an alkyl group having from 2 to 4 carbon atoms.

Specific examples of the polyvalent alcohol-fatty acid ester may include, for example, propylene glycol caprylate, propylene glycol caprate, butylene glycol caprylate, butylene glycol caprate, glycol butyrate, and

propylene glycol butyrate.

The organic solvents as described hereinabove may be employed solely or in admixture with the other organic solvents. The mixture advantageously contains the lower alcohol in the range from approximately 5% to 60% by weight, preferably from approximately 10% to 50% by weight.

The organic solvents may be admixed with the cyclosporin at the rate ranging from approximately 0.5 part to 10 parts by weight, preferably from approximately 1 part to 5 parts by weight, per part by weight of cyclosporin. As the organic solvents, the lower alcohol, particularly ethanol, is preferred. The lower alcohol can serve as a solvent for the cyclosporin as well as acts for accelerating the ability of the cyclosporin to infuse or penetrate through the skin.

The rate of the lower alcohol to be admixed with the cyclosporin may preferably be determined so as to amount to 2% by weight or more with respect to the total weight of the topical preparation, in order to accelerate the ability of the cyclosporin for infusion or penetration through the skin. If the concentration of the lower alcohol increases, the extent of irritation becomes so severer that the concentration of the lower alcohol may be reduced to 15% by weight or lower with respect to the total weight of the topical preparation. It is to be noted, however, that the concentration of the lower alcohol may preferably range from 3% to 6% by weight with respect to the total weight of the topical preparation, in order to focus on improvements in the ability of the cyclosporin for infusion or penetration through the skin and a low degree of irritation.

It is noted that for the topical preparations according to the present invention, it is preferred to use such an organic solvent as having a boiling point of 160° C or higher, preferably 180° C or higher and being sparingly volatile or volatilizable. Such organic solvents may include, for example, a higher aliphatic alcohol having 8 carbon atoms or more and a divalent alcohol-fatty acid ester.

The topical preparations according to the present invention contains the ester of the fatty acid in liquid state at ambient temperature with the monovalent alcohol and/or the alkanol amine. The fatty acid ester with the monovalent alcohol may have 8 carbon atoms or more, preferably 12 carbon atoms or more.

The monovalent alcohol component of the monovalent alcohol-fatty acid esters may be a residue of a straight- or branch-chained aliphatic alcohol having from 1 to 22 carbon atoms, preferably from 2 to 18 carbon atoms. The fatty acid component may be a straight-chained or branch-chained, monovalent or divalent fatty acid having from 4 to 22 carbon atoms, preferably from 6 to 18 carbon atoms. The monovalent alcohol and fatty acid components may in each case contain an unsaturated bond. The monovalent alcohol component thereof may include, for example, ethanol, propanol, isopropanol, butanol, hexanol, octanol, isooctanol, dodecanol, isododecanol, myristyl alcohol, cetyl alcohol, hexadecyl alcohol, 2-ethylhexyl alcohol, 2-octyl dodecanol and the like. The fatty acid component may include, for example, a monovalent fatty acid such as butyric acid, octanoic acid, nonanoic acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolic acid, and erucic acid, and a divalent fatty acid such as succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, sebacic acid, and dodecane diacid. Preferred examples of the fatty acid esters with the monovalent alcohols may include, for example, a monovalent fatty acid ester such as ethyl myristate, isopropyl myristate, isotridecyl myristate, isopropyl laurate, isopropyl caprylate, isopropyl palmitate, isopropyl butyrate, amyl butyrate, and octyl butyrate, and a divalent fatty acid ester such as diethyl succinate, diisopropyl succinate, diethyl adipate, diisopropyl adipate, diisooctyl adipate, dioctyl adipate, didecyl adipate, decyl isooctyl adipate, diethyl azelate, diisopropyl azelate, diisooctyl azelate, diethyl sebacate, diisopropyl sebacate, dibutyl sebacate, and dioctyl sebacate.

Specific examples of the alkanol amines may include, for example, diethanol amine, triethanol amine, isopropanol amine, triisopropanol amine, dibutanol amine, tributanol amine, and the like.

The monovalent alcohol-fatty acid ester and the alkanol amine can serve as improving the ability of the cyclosporin solution in the organic solvents to infuse or penetrate through the skin as well as demonstrate the action of homogeneously dispersing the cyclosporin, dissolved in the organic solvents, in the oily substance in solid form. The rate of these compounds may range usually from approximately 1% to 15% by weight, preferably from approximately 3% to 10% by weight, with respect to the total weight of the topical preparation. Further, these compounds may be employed at the rate ranging usually from approximately 2 parts to 5 parts by weight, preferably from approximately 2.5 parts to 4 parts by weight, with respect to part by weight of the organic solvent or solvents.

The topical preparations according to the present invention contains the oily substance in solid form at ambient temperature. It is noted herein that the term "solid" referred to herein is intended to mean semi-solid as well as solid. The oily substances may include, for example, an alcohol, an fatty acid, an ester, a triglyceride, wax, vaseline, and the like. The alcohol may include, for example, palmityl alcohol, stearyl alcohol, eicosyl alcohol, glycerine, polyglycerin, and the like. The fatty acid may include, for example, palmitic acid, stearic acid, oleic acid, arachic acid, behenic acid, montanic acid, melissic acid, sebacic acid,

and the like. The ester may include, for example, butyl stearate, hexyl laurate, myristyl myristate, dodecyl oleate, 2-octyldodecyl myristate, hexyl decyl octanoate, cetyl lactate, glyceryl caprate, glyceryl capriate, and the like. As the triglyceride, there may be employed a variety of materials originating from sources such as animals or naturally occurring plants or vegetables, which are generally called fats and oils and which can be commercially available. It may include, for example, a large variety of vegetable oils, cow fats, liver fats, lanolin, lard, and the like. Preferable ones are vegetable oils, particularly olive oil, camellia oil, soybean oil, rapeseed oil, corn oil, castor oil, safflower oil, and the like. There may also be employed fish oil rich in eicosapentadecanoic acid that recently draws increasing attention due to its action for allergy or malignant tumor.

The rate of the oily substance is not restricted to a particular one and may be formulated at any arbitrary rate in accordance with the desired properties of the topical preparations. Generally, the rate of the oily substance may range from approximately 1 part to 10 parts by weight, preferably from approximately 2 parts to 8 parts by weight, with respect to part by weight of the total weight of the organic solvent and the monovalent alcohol-fatty acid ester and/or the alkanol amine, which is in liquid state at room temperature.

The surfactant is contained in the topical preparations according to the present invention. As the surfactants, there may be employed a variety of surfactants, including anionic, cationic, non-ionic or amphoteric ones. The non-ionic surfactants may preferably be employed in terms of a low degree of irritation to the skin. As the non-ionic surfactants, there may be mentioned, for example, an ethylene oxide type surfactant, a polyhydroxy type surfactant, a polymer type surfactant, and the like. The ethylene oxide type surfactants may include, for example, an ethylene oxide adduct of a higher alcohol, an ethylene oxide adduct of a higher fatty acid, an ethylene oxide adduct of an alkyl phenol, an ethylene oxide adduct of an fatty acid amine, an ethylene oxide adduct of an fatty acid amide, an ethylene oxide adduct of a polyvalent alcohol, an ethylene oxide/propylene oxide block copolymer, and the like. The polyhydroxy type surfactants may include, for example, a glycerin monofatty acid ester, a pentaerythritol fatty acid ester, a sorbitan fatty acid ester, a sucrose fatty acid ester, an fatty acid amide of ethanol amine and an alkylene oxide adduct thereof, and the like. Among these polyhydroxy type surfactants, there may be advantageously employed a polyoxy ethylene sorbitan fatty acid ester, a polyoxy ethylene glyceryl monofatty acid ester, a polyoxy propylene monofatty acid ester, the sorbitan fatty acid ester, a polyoxy ethylene alcohol ether, and the like. These surfactants may be employed solely or in admixture with the other surfactant or surfactants.

The amount and the rate of the surfactant is not restricted to a particular one and may vary depending upon the desired properties of the topical preparation, although the surfactant may be generally contained in the range of from approximately 5% to 50% by weight, preferably from approximately 20% to 45% by weight, with respect to the total weight of the topical preparation in the case of the topical preparation being of a non-emulsion type and from approximately 1% to 20% by weight, preferably from approximately 5% to 15% by weight, with respect to the total weight thereof in the case of the topical preparation being of an emulsion type.

The topical preparation in accordance with the present invention may, as desired, contain an additive such as a filler, an aid for dissolving cyclosporin, a thickening agent, a colorant, a flavor, water, liquid paraffin, squalane, an emulsification stabilizer, a bactericide, a fungicide, and the like. The filler may be finely divided powder of an organic type or of an inorganic type. The particle size of the filler may range usually from approximately 0.1 μm to 20 μm, preferably from approximately 0.5 μm to 10 μm. Appropriate examples of the fillers may include silica, alumina, titania, resin powder, silicate powder, clay powder, sepiolite powder, mommorilonite powder, fluorinated mica powder, hydroxypropyl cellulose powder, and the like. The aid of dissolving cyclosporin may include, for example, an alkylene glycol and a polyalkylene glycol such as ethylene glycol, propylene glycol, isopropylene glycol, polyethylene glycol, polypropylene glycol, and the like. The rate and the amount of the dissolving aid may range from approximately 0.2 part to 5 parts by weight with respect to part of the total weight of the organic solvent. The alkylene glycol serves as accelerating the infusion or penetration of the cyclosporin through the skin.

The topical preparations according to the present invention may be applied in the form of an emulsion or a non-emulsion. When the topical preparations are formulated in a non-emulsion form, they may preferably comprise the following composition:

a. Cyclosporin: from approximately 0.1% to 10% by weight, preferably from approximately 1% to 7% by weight;

b. Organic solvent: from approximately 1% to 40% by weight, preferably from approximately 2% to 20% by weight;

c. Monovalent alcohol-fatty acid ester in liquid state at ambient temperature and/or the alkanol amine: from 1% to 15% by weight, preferably from approximately 3% to 10% by weight;

d. Oily substance in solid state at ambient temperature: from approximately 20% to 80% by weight, preferably from approximately 35% to 60% by weight;

e. Surfactant: from approximately 5% to 50% by weight, preferably from approximately 20% to 45% by weight; and

f. Filler: from 0% to approximately 15% by weight, preferably from approximately 5% to 10% by weight.

When the lower alcohol is employed solely as the organic solvent for the topical preparation of the non-emulsion type, the lower alcohol may conveniently be contained at a rate ranging from approximately 2 to 15% by weight, preferably from approximately 3% to 10% by weight. In this case, the surfactant may conveniently be contained at a rate ranging from approximately 20% to 45% by weight, preferably from approximately 20% to 40% by weight and the oily substance may conventionally be contained at a rate in the range of from approximately 35% to 60% by weight, preferably from approximately 40% to 55% by weight. Further, the surfactant to be employed may have an HLB of 8 to 25, preferably from 9 to 12.

The topical preparation of the non-emulsion type may be formulated by mixing a cyclosporin solution in the organic solvent and the monovalent alcohol-fatty acid ester in liquid state at ambient temperature and/or the alkanol amine, mixing the resulting mixture with the oily substance and the surfactant, and adding the filler to the resulting mixture as needed, and then homogenizing the mixture.

The topical preparations in accordance with the present invention in an emulsion form may preferably comprise the composition as follows:

a. Cyclosporin: from approximately 0.1% to 10% by weight, preferably from approximately 1% to 7% by weight;

b. Organic solvent: from approximately 1% to 20% by weight, preferably from approximately 2% to 12% by weight;

c. Monovalent alcohol-fatty acid ester in liquid state at ambient temperature and/or the alkanol amine: from 1% to 15% by weight, preferably from approximately 3% to 10% by weight;

d. Oily substance in solid state at ambient temperature: from approximately 10% to 35% by weight, preferably from approximately 15% to 30% by weight;

e. Surfactant: from approximately 1% to 20% by weight, preferably from approximately 5% to 15% by weight;

f. Filler: from 0% to approximately 10% by weight, preferably from approximately 0.1% to 5% by weight; and

g. Sterilized water: from approximately 30% to 75% by weight, preferably from approximately 40% to 50% by weight.

The topical preparations in the form of an emulsion may be prepared by mixing the components (a) to (f), inclusive, at elevated temperature to give an oily mixture in a liquid state, referred to hereinafter as "mixture A", and adding sterilized pure water, referred to hereinafter as "water B" to the mixture A with stirring at elevated temperature. The water B may be added at a rate of from approximately 30% to 75% by weight with respect to the total weight of the mixture A and the water B. To the water B may in advance be added an aid of infusion or penetration of cyclosporin through the skin, a viscosity adjusting agent, the bactericide, a water-soluble substance such as an alkanol amine. The infusion or penetration aid may include, for example, an alkylene glycol such as ethylene glycol, propylene glycol, butylene glycol, and the like. The viscosity adjusting agent may include, for example, a polyalkylene glycol such as polyethylene glycol, polypropylene glycol, and the like; a polyvalent alcohol such as glycerin and the like; and a water-soluble polymer such as carboxyvinyl polymer and the like. The topical preparations in the emulsion form may be of an oil/water type and of a water/oil type. For the topical preparations of the oil/water type, the surfactant having an HLB of 9 to 18 may preferably be employed; for the topical preparations of the water/oil type, the surfactant having an HLB of 2 to 8 may preferably be employed. To the topical preparations of the emulsion type may be added, as needed, a viscous oily substance such as liquid paraffin, glycerin, vaseline, and the like.

The topical preparations according to the present invention may be administered by applying them directly to the affected part of the skin or by applying them in the form of a patch, plaster, poultice, or the like to the affected part thereof, several times, e.g. once to thrice, per day. The number of applications may appropriately be increased or reduced depending upon the extent of the disease to be applied.

In accordance with the topical preparations of the present invention, a mixture of the cyclosporin solution in the organic solvent with the liquid monovalent alcohol-fatty acid ester and/or alkanol amine is contained in the oily substance in homogeneously dispersed manner. Hence, the topical preparations is so highly likely to infuse or penetrate through the skin that they can demonstrate highly therapeutic effects upon autoimmune or allergic skin diseases merely by applying them to the affected part of the skin. Further, the topical preparations are little irritative or extremely low in irritation to the skin so that they are highly

safe.

The topical preparations according to the present invention are highly effective for the therapy of various dermal diseases such as atopic dermatitis, psoriasis, contact dermatitis, allergic contact dermatitis, alopecia, and the like. Further, they are effective for treating other dermal diseases, such as scald. The topical preparations can assist adapt a skin piece grafted to the site of skin grafting.

The present invention will be described more in detail by way of examples.

Example 1:

For a topical preparation, there were employed the components as follows:

Cyclosporin:	1% by weight
95% Ethanol:	3% by weight
Isopropyl myristate:	5% by weight
Olive oil:	48% by weight
Polyoxyethylene (5) glyceryl monostearate:	35% by weight
Finely divided silica (Aerosil 200)	8% by weight

The topical preparation was formulated by mixing isopropyl myristate, polyoxyethylene (5) glyceryl monostearate and olive oil with stirring at 50° C to give a homogenous solution to which a solution of cyclosporin in ethanol was added, and the resulting mixture heated to 30° - 35° C was mixed with aerosil to give an ointment.

Example 2:

A topical preparation was prepared in substantially the same manner as in Example 1 using the components as follows:

Cyclosporin:	1% by weight
95% Ethanol:	5% by weight
Isopropyl myristate:	5% by weight
Olive oil:	47% by weight
Polyoxyethylene (5) glyceryl monostearate:	35% by weight
Finely divided silica (Aerosil 200)	7% by weight

Example 3:

A topical preparation was prepared in substantially the same manner as in Example 1 using the components as follows:

Cyclosporin:	2% by weight
95% Ethanol:	10% by weight
Isopropyl myristate:	5% by weight
Camellia oil:	44% by weight
Polyoxyethylene (5) glyceryl monostearate:	32% by weight
Finely divided silica (Aerosil 200)	7% by weight

Example 4:

After the skins of guinea pigs were sensitized with dinitrofluorobenzene (DNFB), DNFB were applied again, thereby causing the strong allergic reaction to emerge on the skins of the guinea pigs.

The efficacy of the topical preparations according to the present invention was observed with this experimental model.

Cyclophosphamide was intraperitoneally administered at the rate of 200 mg per kg three days before the sensitization of male Hartley guinea pigs, weighing from 40 grams to 500 grams, and 50 µl of a 10% DNFB solution in a 1:1 mixture of acetone and olive oil) was applied to one earlobe of each of the guinea pigs. At day 8, a dose of 20 µl of 0.5% or 0.1% DNFB solution in a 4:1 mixture of acetone and olive oil was applied to the both sides of the depilated abdominal portions of the guinea pigs, whereby contact dermal allergic reaction was induced.

After DNFB was then applied as an antigen to the corresponding sites of the both abdominal portions, the topical preparations prepared in Example 1 (containing cyclosporin at the rate of 0.1%, 1% and 10%) were applied in the amount of 50 µl thereto. This application was repeated twice a day at an interval of 8 hours. The first application of each topical preparation was conducted immediately after DNFB had been air dried.

The allergic reaction was evaluated at 24 hours, 48 hours and 72 hours after the application of the antigen in accordance with the following criteria: Rating 4 = swell in red; rating 3 = colored in red; rating 2 = colored in pink; rating 1 = a spot colored in pink; and rating 0 = no change. The values as shown in Table 1 below represent the mean value plus or minus the standard error (SE).

The statistical treatment was conducted with Student's t-test, and a significant difference was justified if the error rate was $p<0.05$.

The application of the 0.5% DNFB solution caused the strongest allergic reaction over the time range from 24 hours to 48 hours after the application. The 0.1% cyclosporin ointment suppressed the allergic reaction to a considerable extent with no significant difference. On the other hand, the ointment containing 1% cyclosporin reduced the allergic reaction to a remarkable extent at 24 hours with the significant difference of $p<0.01$. Even at 48 hours and 72 hours, the allergic reaction was suppressed with the significant difference. Further, the ointment containing 10% cyclosporin demonstrated the significant suppression of the allergic, like the 1% cyclosporin ointment. As a control, the ointment base only did not suppress the allergic reaction at all. The results are shown in Table 1 below.

TABLE 1

Test Samples		24 hours	48 hours	72 hours
Cyclosporin (%)	No. of guinea pigs			
0	9	3.4 ± 0.2	3.4 ± 0.2	2.7 ± 0.2
0.1	9	2.4 ± 0.3	2.7 ± 0.3	1.8 ± 0.3
1.0	9	0.7 ± 0.3**	1.0 ± 0.3**	1.0 ± 0.3**
0	4	3.3 ± 0.3	3.3 ± 0.3	3.3 ± 0.3
10	4	0.8 ± 0.5*	1.0 ± 0.6*	1.0 ± 0.5

* $p < 0.05$
** $p < 0.01$

When the 0.1% DNFB solution was applied, the strongest allergic reaction was caused to appear at 48 hours after the application. The 0.1% cyclosporin topical preparation suppressed the allergic reaction to a remarkable extent with the significant difference of $p < 0.01$. The allergic reaction was likewise suppressed even at 48 hours and 72 hours. On the other hand, the topical preparations containing 1% and 10% cyclosporin showed the reduction in the allergic reaction with the significant difference, like the topical preparation containing 0.1% cyclosporin. As a control, the ointment base only did not suppress the allergic reaction at all. The results are shown in Table 2 below.

TABLE 2

Test Samples		24 hours	48 hours	72 hours
Cyclosporin (%)	No. of guinea pigs			
0	8	2.1 ± 0.3	3.1 ± 0.2	2.5 ± 0.2
0.1	8	0.3 ± 0.2**	1.0 ± 0.2**	0.8 ± 0.2**
1.0	8	0.1 ± 0.1**	0.4 ± 0.3**	0.1 ± 0.1**
0	4	2.0 ± 0.4	3.0 ± 0	2.3 ± 0.3
10	4	0 ± 0*	0.5 ± 0.3*	0.3 ± 0.3*

* $p < 0.05$
** $p < 0.01$

Example 5:

Case 1:

A male patient, 27 years old, has been affected with atopic dermatitis since his age of 22 although a temporary remission had been gained at his age of 8 years from the atopic dermatitis since his age of 3. Various steroidal ointments were applied so far; they were found hardly effective. With the 10% cyclosporin ointment according to the present invention, an itch on his skin disappeared four to five hours after the application of the ointment and the lichenized erythra peculiar in the atopic dermatitis disappeared completely at day 3 after its application when the ointment was applied twice per day.

Case 2:

A male child, 6 years old, has been affected with atopic dermatitis since his age of 3 and was administered with Azeptin, Zaditen, and Rizaben as well as ointments such as Rinderon V, Locorten and Methaderm; however, no effect was recognized. The application of a 5% cyclosporin ointment according to the present invention eliminated an itch to his skin within 5 hours after the topical administration and the itch, erythema and wet erosion of the affected part had disappeared within 24 hours after the application thereof.

Case 3:

A male patient, 52 years old, was affected with psoriatic arthritis, and the 1% cyclosporin ointment according to the present invention was applied to the wet erythema with a clear borderline and the scales on the surface thereof. The 1% cyclosporin ointment improved the Auspitz phenomenon within 24 hours after the application with the erythema disappearing at day 3 from the application of the ointment.

Example 6:

In order to demonstrate the efficacy of the topical preparations according to the present invention, the ointments were prepared from the components as shown in Table 3 below and the efficacy thereof was evaluated in substantially the same manner as in Example 4. The evaluation results are shown in Table 3 below.

TABLE 3

Components	Contents (% by weight)						
	Experiment Nos.						
	1*	2*	3	4	5	6	7
Cyclosporin	5	5	5	5	10	5	10
95% Ethanol	0	0	2	5	10	5	10
Isopropyl myristate	5	5	5	5	5	0	3
Olive oil	48	48	48	45	35	36	36
Polyoxyethylene glycol monostearate	35	35	35	35	35	36	36
Aerosil	5	7	5	5	5	6	6
Triethanol amine	2	0	0	0	0	3	2
Efficacy	None	None	Yes	Yes	Yes	Yes	Yes

* Comparative Examples

Comparative Examples:

The following topical preparations containing cyclosporin were prepared for comparative purposes in conventional manner:

i. A caster oil suspension containing 5% by weight of cyclosporin;

ii. A suspension of 5% by weight of cyclosporin in castor oil containing 20% by weight of propylene glycol; and

iii. An ointment containing 10% by weight of cyclosporin, 43% by weight of olive oil, 10% by weight of ethanol, 7% by weight of polyoxyethylene (5) oleate, and 30% by weight of silicon dioxide in colloidal state.

The topical preparations prepared in the manner as described hereinabove were evaluated for their pharmaceutical efficacy in substantially the same manner as in Example 4; however, none of them were found significantly effective.

Example 7:

For a topical preparation, there were employed the components as follows:

Cyclosporin:	5% by weight
95% Ethanol:	2% by weight
Isopropyl myristate:	7% by weight
Camellia oil:	40% by weight
Polyoxyethylene (5) glyceryl monostearate:	41% by weight
Finely divided silica (Aerosil 200)	5% by weight

The topical preparation was formulated in substantially the same manner as in Example 1.

Example 8:

A topical preparation was prepared in substantially the same manner as in Example 1 using the components as follows:

Cyclosporin:	5% by weight
95% Ethanol:	5% by weight
Isopropyl myristate:	5% by weight
Camellia oil:	39% by weight
Polyoxyethylene (5) glyceryl monostearate:	39% by weight
Finely divided silica (Aerosil 200)	5% by weight

Example 9:

After each of the topical preparations prepared in Examples 7 and 8 were stored in a closed state for 6 months at relative temperature of 75% and temperature of 40° C, the content of cyclosporin within the topical preparation was measured. As a result, it was found that no substantial changes were observed between before and after storage. Thus, it is confirmed that cyclosporin is sustained in a stable state for a long period of time.

Example 10:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 70 grams of 2-octyl dodecanol, 30 grams of isopropyl myristate, 20 grams of isotridecyl myristate, 10 grams of polyoxyethylene sorbitan monooleate (20), 50 grams of polyoxyethylene glyceryl monostearate (5), 10 grams of sorbitan monostearate, 30 grams of cetanol, 40 grams of sebacate and 30 grams of olive oil at 80 C. On the other hand, a mixture (B) was prepared by adding 30 grams of propylene glycol, 20 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 1 gram of methyl p-hydroxybenoate, and 1 gram of propyl p-hydroxybenzoate to 596 ml of sterilized water and heating the mixture to approximately 82° C. As the two mixtures reached the predetermined temperatures, the mixture A was gradually added with vigorous stirring to the mixture B, thereby producing an emulsion. After the addition was completed, the heating was ceased and the temperature of the emulsion was stirred and cooled down to 60° - 55°. Then, sterilized water was added to make the total volume of the mixture 1 kg. The whole mixture was allowed to stand and defoamed, followed by filling in a vessel.

In the above composition, polyoxyethylenel glyceryl monostearate (5) can be replaced by 2.0% by weight of polyoxyethylene (2) cetyl ether; sorbitan monostearate can be replaced by squalane SK; and cetanol can be replaced by behenyl alcohol. Further, the total volume of the sterilized water used for the mixture (B) can be replaced by liquid paraffin.

Example 11:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 50 grams of ethanol, 50 grams of isopropyl myristate, 50 grams of polyethylene glycol (400), 30 grams of dithyl sebacate, 80 grams of olive oil, 30 grams of polyoxyethylene monostearate (5), 30 grams of polyethylene glycol monostearate (40), and 20 grams of sorbitan monostearate at elevated temperature. On the other hand, a mixture (B) was prepared by dissolving 50 grams of polythylene glycol, 20 grams of diisopropanol amine, 10 grams of carboxyvinyl polymer, 1 gram of methyl p-hydroxybenoate, and 1 gram of propyl p-hydroxybenzoate in 528 ml of sterilized water at elevated temperature. The mixture A was gradually added with vigorous stirring to the mixture B, thereby producing an emulsion. After the addition was completed, the total volume of the mixture was increased to make 1 kg by adding sterilized water to the mixture.

In the above composition, ethanol can be replaced by behenyl alcohol, and diisopropanol amine can be replaced by triethanol amine.

Example 12:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 10 grams of octyl alcohol, 50 grams of olive oil, 30 grams of isopropyl myristate, 25 grams of isotridecyl myristate, 20 grams of polyoxyethylene sorbitan monooleate (20), 60 grams of polyoxyethylenel glyceryl monostearate (5), 20 grams of sorbitan stearate, 30 grams of cetanol, 25 grams of stearic acid, and 35 grams of diethyl sebacate at 80° C. On the other hand, a mixture (B) was prepared by adding and dissolving 20 grams of polythylene glycol, 20 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 0.5 gram of methyl p-hydroxybenoate, and 0.5

gram of propyl p-hydroxybenzoate to and in approximately 400 ml of sterilized water by heating the mixture to 82° C or higher. The mixture B was gradually added with vigorous stirring to the mixture A, thereby producing an emulsion. After the addition was completed, the heating was ceased and sterilized water was added at 80° C to the resulting mixture with stirring at room temperature, thereby increasing the total volume of the mixture to make 1 kg. The whole mixture was allowed to stand and defoamed; then the ointment in cream form was filled in a container.

In the above composition, isopropyl myristate can be replaced by isopropyl palmitate.

Example 13:

A mixture (A) was prepared by dissolving 50 grams of cyclosporin, 30 grams of bees wax, 80 grams of 2,6-dimethyl-4-heptanol, 30 grams of olive oil, 40 grams of isotridecyl myristate, 20 grams of polyoxyethylene solbitol hexastearate (20), 60 grams of polyoxyethylenel glyceryl monostearate (5), 20 grams of polyoxyethylene (60) hardened castor oil, 40 grams of cetostearyl alcohol, and 40 grams of diethyl sebacate at 80° C. On the other hand, a mixture (B) was prepared by adding and dissolving 30 grams of polyethylene glycol, 20 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 0.5 gram of methyl p-hydroxybenoate, and 0.5 gram of propyl p-hydroxybenzoate to and in 510 ml of sterilized water by heating the mixture to 82° C or higher. The mixture (B) was gradually added with vigorous stirring to the mixture (A) maintained at 80° C. After the addition was completed, the heating was ceased and the mixture was cooled down to 60° - 55° C with stirring. Then, sterilized water heated to 80° C was added to the resulting mixture with stirring at room temperature, thereby increasing the total volume of the mixture to make 1 kg. The whole mixture was allowed to stand and defoamed; then the resulting mixture was filled in a container.

In the above composition, the bees wax can be replaced by polyoxyethylene lanolyl alcohol or a bees wax derivative; isotridecyl myristate can be replaced by 0.2% by weight of silicone oil; polyoxyethylenel sorbitan oleate (20) can be replaced by polyoxyethylenel sorbitan-fatty acid ester; sorbitan monostearate can be replaced by squalane SK; and sterilized water can be replaced by liquid paraffin.

Example 14:

A mixture (A) was prepared by mixing 50 grams of cyclosporin, 80 grams of propylene glycol monocaprylate, 30 grams of isopropyl myristate, 30 grams of PEG monostearate (25EO), 30 grams of polyethylene glycol, 20 grams of isotridecyl myristate, 20 grams of cetanol, 50 grams of olive oil, 80 grams of whale wax, 30 grams of sorbitan monostearate, 30 grams of polyoxyethylene glyceryl monostearate (5), 30 grams of stearic acid, 20 grams of diisopropanol amine, and 40 grams of diethyl sebacate and heating the resulting mixture at 80° C. On the other hand, a mixture (B) was prepared by adding 30 grams of propylene glycol, 15 grams of diisopropanol amine, 2 grams of carboxyvinyl polymer, 0.5 gram of methyl p-hydroxybenoate, and 0.5 gram of propyl p-hydroxybenzoate to approximately 400 ml of sterilized water and heating the resulting mixture to 82° C or higher. The mixture (B) was gradually added with vigorous stirring to the mixture (A) maintained at 80° C or higher. After the addition was completed, the heating was ceased and the mixture was stirred to cool the temperature of the mixture to 60° - 55° C, followed by adding sterilized water heated at 80° C to the resulting mixture to increase the total volume of the mixture to make 1 kg. The whole mixture was allowed to stand and defoamed; then the resulting mixture was filled in a container.

Example 15:

A mixture (A) was prepared by mixing 50 grams of cyclosporin, 70 grams of 2-octyl dodecanol, 30 grams of isoprene glycol, 40 grams of diethyl sebacate, 30 grams of isopropyl myristate, 30 grams of isotridecyl myristate, 60 grams of whale wax, 30 grams of cetanol, 40 grams of stearic acid, 20 grams of POE (5) glyceryl monostearate, 20 grams of PEG monostearate (40EO), 10 grams of sorbitan monostearate, 50 grams of olive oil, and 1 gram of propylparaben and heating the mixture to 80° C. On the other hand, a mixture (B) was prepared by adding 30 grams of butylene glycol, 20 grams of diisopropanol amine, and 1 gram of methylparaben to 460 ml of sterilized water and heating the resulting mixture to 82° C or higher. The mixture (B) was gradually added with vigorous stirring to the mixture (A) maintained at 80° C or higher. After the addition was completed, the heating was ceased and the temperature of the mixture was cooled down to 60° - 55° C with stirring. Then, sterilized water headed at 80° C was added to the resulting mixture, thereby increasing the total volume of the mixture to make 1 kg. The whole mixture was allowed to

stand and defoamed; then the resulting mixture was filled in a container.

Example 16:

A solution of 50 grams of cyclosporin in 80 grams of 2-octyl dodecanol was added to a warmed mixture of 40 grams of isopropyl myristate, 370 grams of olive oil, 378 grams of polyoxyethylene (5) glyceryl monostearate, 2 grams of polyoxyethylene (9) lauryl ether, and 10 grams of sorbitan monostearate, and 70 grams of aerosil was added to the resulting mixture, thereby yielding a topical preparation of a non-emulsion type.

Example 17:

The efficacy of the creamy ointment containing cyclosporin, prepared in substantially the same manner as in Example 15, was confirmed by applying it to the transplant of the skin sections of mice.

The skin sections of 10 male CBA mice of 5 weeks were transplanted to male C3H/HeN mice of the same week. To the transplanted sites and the portions surrounding them was applied approximately 0.1 gram of the ointment prepared in Example 12 two times per day until the transplanted sections eventually fell off. The results are shown in Table 4 below.

TABLE 4

Cyclosporin (%)	Period of Transplantation*	Effect of Extension	Significant Difference**
5.0	>60	>397	$p < 0.001$
1.0	31.3 ± 1.43	207	$p < 0.001$
0.0 (as control)	15.1 ± 0.78	100	-

* mean value ± SE

** Student's t-test

For a control group in which a cream without cyclosporin was applied, the transplanted skin specimens fell off for an average period of transplantation of 12.7 days, while a group in which a cream containing 5% cyclosporin was applied had all the transplanted skin sections grow for 60 days or longer. For a group in which a cream containing 1% cyclosporin was applied, the period of transplantation for which the transplanted skin sections grew was extended with significant difference to mean 31.3 days.

Example 18:

Eight Hartley male guinea pigs weighing approximately 300 grams were intraperitoneally administered with 150 mg/kg of cyclophosphamide, and 50 µl of a 10% dinitroflurobenzene (DNFB) solution was applied to one earlobe of each guinea pig in three days after the intraperitoneal administration. The DNFB solution was prepared by dissolving the predetermined amount of the DNFB in a 1:1 mixture of acetone with olive oil. After 8 days, the hairs on the both abdominal parts were cut off and 20 µl of a 0.1% DNFB solution was applied to the depilated abdominal parts of the guinea pigs to induce contact dermal allergy. Immediately thereafter, the cyclosporin ointment prepared in substantially the same manner as in Example 15 was applied to the parts to which the DNFB solution was applied, followed by applying the cyclosporin ointment thereto in 8 hours. To a control group, the base used in Example 15 without cyclosporin was applied in accordance with the same schedule as described hereinabove.

The allergic reaction was determined in 24 hours, 48 hours and 72 hours after the application of the DNFB solution as the antigen, and the rating was: 4 = swell in red; 3 = colored in red; 2 = inflammation causing the skin to turn pink; 1 = inflammation causing the skin to turn pale pink; and 0 = no change. The results are shown in Table 5 below.

TABLE 5

Cyclosporin (%)	Severity of Dermal Reaction (mean value ± SE)		
	24 hours	48 hours	72 hours
1.0	0.0 ± 0.0**	0.3 ± 0.2**	0.1 ± 0.1**
0.1	0.3 ± 0.3**	0.9 ± 0.2**	0.7 ± 0.3**
0.0 (control)	2.2 ± 0.3**	3.1 ± 0.2**	2.4 ± 0.2**

** $p < 0.001$ in Student's t-test

In these experiments, the strongest allergic reaction was induced over the range extending from 24 hours to 48 hours after the application of the DNFB solution. The ointment containing 1.0% cyclosporin strongly suppressed the allergic reaction and the ointment containing 0.1% cyclosporin suppressed the allergic reaction with significant difference.

Claims

1. A topical preparation comprising (a) cyclosporin; (b) an organic solvent in which said cyclosporin is to be dissolved; (c) an ester of a fatty acid with a monovalent alcohol having a total number of carbon atoms of 8 or more and/or an alkanol amine, each being in liquid state at 25° C; (d) an oily substance in solid state at 25° C; and (e) a surfactant; wherein said cyclosporin is contained at a rate ranging from approximately 0.1% to 10% by weight, and said ester and/or said alkanol amine are/is contained at a rate ranging from approximately 1% to 15% by weight.

2. A topical preparation as claimed in claim 1, wherein said organic solvent is an aliphatic alcohol in liquid state at 25° C.

3. A topical preparation as claimed in claim 2, wherein said aliphatic alcohol is a lower alcohol.

4. A topical preparation as claimed in claim 3, wherein said lower alcohol is ethanol.

5. A topical preparation as claimed in claim 2, wherein said aliphatic alcohol is a higher alcohol having a branched chain and carbon atoms of 8 or more.

6. A topical preparation as claimed in claim 5, wherein said higher alcohol is 2-octyldodecanol.

7. A topical preparation as claimed in claim 1, wherein said organic solvent is a fatty acid monoester with a polyvalent alcohol, having liquid state at ambient temperature.

8. A topical preparation as claimed in claim 7, wherein said monoester is propyleneglycol monocaprate or propylene glycol monocaprylate.

9. A topical preparation as claimed in any one of claims 1 to 8, wherein said organic solvent is contained at a rate ranging from approximately 0.5 part to 10 parts by weight with respect to part by weight of said cyclosporin.

10. A topical preparation as claimed in any one of claims 1 to 9, wherein said fatty acid ester with said monovalent alcohol is an ester of a monovalent fatty acid having carbon atoms of 8 or more.

11. A topical preparation as claimed in any one of claims 1 to 9, wherein said fatty acid ester with said monovalent alcohol is a diester of a divalent fatty acid having carbon atoms of 4 or more.

12. A topical preparation as claimed in any one of claims 1 to 9, wherein said fatty acid ester with said monovalent alcohol is a myristic acid ester and/or a sebacic acid diester.

13. A topical preparation as claimed in any one of claims 1 to 12, wherein said oily substance is at least one member selected from a group consisting of a fatty acid having a melting point of 25° C or higher, an alcohol, an ester and a triglyceride.

14. A topical preparation as claimed in claim 13, wherein said triglyceride is vegetable oil.

15. A topical preparation as claimed in any one of claims 1 to 14, wherein said surfactant is a non-ionic surfactant.

16. A topical preparation as claimed in any one of claims 1 to 15, further comprising a filler.

17. A topical preparation as claimed in any one of claims 1 to 16, further comprising an alkylene glycol and/or a polyalkylene glycol.

18. A topical preparation comprising (a) cyclosporin; (b) a lower alcohol; (c) an ester of a fatty acid with a monovalent alcohol having a total number of carbon atoms of 8 or more and/or an alkanol amine, each being in liquid state at 25° C; (d) an oily substance in solid state at 25° C; and (e) a surfactant; wherein said cyclosporin is contained at a rate ranging from approximately 0.1% to 10% by weight, said lower alcohol is contained at a rate ranging from approximately 2% to 15% by weight, and said ester and/or said alkanol amine are/is contained at a rate ranging from approximately 1% to 15% by weight.

19. A topical preparation as claimed in claim 18, wherein said lower alcohol is selected from at least one member selected from a group consisting of ethanol, isopropanol, propanol and isobutanol.

20. A topical preparation as claimed in claim 18 or 19, wherein said fatty acid ester with said monovalent alcohol is an ester of a straight-chained or branched-chain fatty acid having from 8 to 24 carbon atoms.

21. A topical preparation as claimed in any one of claims 18 to 20, wherein said oily substance is vegetable oil.

22. A topical preparation as claimed in any one of claims 18 to 21, wherein said surfactant is a non-ionic surfactant.

23. A topical preparation as claimed in any one of claims 18 to 22, further comprising a filler at a rate ranging from approximately 5% to 10% by weight.

24. A topical preparation comprising 0.1% to 10% by weight of cyclosporin; 2% to 15% by weight of ethanol; 1% to 15% by weight of isopropyl myristate; 35% to 60% by weight of olive oil or camellia oil; 20% to 40% by weight of a surfactant; and 5% to 10% by weight of silica.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP92/00798

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

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II. FIELDS SEARCHED

Minimum Documentation Searched [7]

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IPC	A61K37/02, 9/06

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts 1967 - 1992

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 3-109332 (Shiseido Co., Ltd.), May 9, 1991 (09. 05. 91), Claim, lower left column, page 4	1-24
A	JP, A, 2-121929 (Sand AG.), May 9, 1990 (09. 05. 90), Claim, upper right column, page 6 & GB, A, 2222770 & DE, A, 3930928 & FR, A, 2636534 & AU, A, 8941400 & CH, A, 679118 & ZA, A, 8907066	1-24
A	JP, A, 2-17127 (Sand AG.), January 22, 1990 (22. 01. 90), Claim & GB, A, 2218334 & DE, A, 3915617 & FR, A, 2631235 & CH, A, 679119	1-24

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

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International Searching Authority	**Signature of Authorized Officer**
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(54) **Oil-in-water microemulsions**

(57) Water-insoluble pharmaceutically active substances such as cyclosporin are formulated for administration in the form of an oil-in-water microemulsion, wherein the active substance is fully dissolved in the dispersed oil particles. The oil is C_8 to C_{20} fatty acid vegetable oil glycerides, and lecithin and another surfactant are included to form and stabilise the microemulsion in which the hydrophilic phase comprises propylene glycol. A preconcentrate comprising the above components but free from any hydrophilic phase can be utilised to make up the compositions, which are most suitably soft gelatine capsules or oral administration fluids. The glycerides are preferably from castor oil, coconut oil or peanut oil.

EP 0 760 237 A1

Description

This invention relates to pharmaceutical compositions for the administration of water-insoluble pharmaceutically active substances.

There are a number of pharmaceutically active substances which are water-insoluble and which, as a result, present a number of problems for their safe administration and bioavailability. Among such substances are the cyclosporins, and water-insoluble peptides, antimicrobials and antineoplastics, for example. There have been many proposals of pharmaceutical formulations for the administration of the cyclosporins, some of which are described in the following patent specifications: WO92/09299, GB-A-2015339, GB-A-2270842, WO94/08610, WO92/18105, GB-A-2228198, US-A-4388307, GB-A-2222770, EP-A-0539319 and EP-A-0589843.

In general, because the cyclosporins are hydrophobic, pharmaceutical compositions containing them usually comprise lipophilic materials such as oils. GB-A-2228198 describes, for example, pharmaceutical compositions containing cyclosporin in a carrier medium of a fatty acid triglyceride, a glycerol fatty acid partial ester or propylene glycol or sorbitol complete or partial ester, and a surface active agent having an HLB of at least 10. These oil-based compositions are not intended to be emulsified in water but are used as such, and are preferably free of ethanol.

Other cyclosporin compositions are known which contain not only hydrophobic oils but also hydrophilic materials such as propylene glycol and ethanol in which cyclosporins are soluble. There compositions are in the form of emulsions. Emulsions have certain advantages over oil-based single phase compositions, and EP-A-0589843 describes some cyclosporin emulsion compositions containing, as the carrier medium, a hydrophilic organic solvent, a mixed mono-, di- and tri-glyceride or a transesterified and polyethoxylated vegetable oil, a polyoxyethylene sorbitan-fatty acid ester surfactant, and an aqueous phase. The carrier medium with the cyclosporin but without the aqueous phase is described as an emulsion preconcentrate.

In recent times, microemulsions have been developed for cyclosporin administration and these have provided provided further advantages over the prior known (coarse) emulsions, especially for oral administration formulations. It is also known to provide so-called "microemulsion preconcentrates". For example, GB-A-2222770 describes a pharmaceutical microemulsion preconcentrate composition comprising cyclosporin, a hydrophilic phase, a lipophilic phase and a surfactant. This preconcentrate is converted to a microemulsion by adding water or another suitable aqueous medium.

These and other microemulsions for cyclosporin are all made by dissolving the cyclosporin in a hydrophilic phase e.g. propylene glycol, and then mixing the solution with the oil and eventually with an aqueous phase. We have found that there can be a tendency with these microemulsions for solid microfine cyclosporin to be formed during their use, e.g. after administration. This is basically undesirable, and we have now found that microemulsions can be made in which this tendency is very much reduced or totally absent.

In particular, we have found that if the water-insoluble active substance is first dissolved directly in the lipophilic phase, rather than in a hydrophilic phase, and then the oil-in-water microemulsion produced therefrom, the substance remains in solution in the lipophilic (oil) phase. This phase is distributed throughout the aqueous phase of the microemulsions as very tiny particles, and it appears that in this way the substance can be taken up very easily and efficiently by the body. In addition, there is no precipitation of the substance out of the oil solution.

In one aspect the present invention provides a pharmaceutical composition in the form of a stable oil-in-water microemulsion, which composition comprises

a) a water-insoluble pharmaceutically active material;
b) C_8 to C_{20} fatty acid mono-, di, or tri-glycerides from a vegetable oil or any mixture of two or more thereof;
c) a phospholipid and another surfactant; and
d) a hydrophilic phase; wherein component (a) has been wholly directly dissolved in component (b), component (b) is dispersed as tiny particles in component (d), and the composition is free from ethanol.

The invention also provides a preconcentrate for mixture with a hydrophilic phase to form a microemulsion of the invention, the preconcentrate composition comprising:

a) a water-insoluble pharmaceutically active material;
b) a C_8 to C_{20} fatty acid mono-, di-, or tri-glyceride from a vegetable oil or any mixture of two or more thereof; and
c) a phospholipid and another surfactant;

wherein component (a) is directly dissolved in component (b), and component (c) is such that, upon mixing the composition with a hydrophilic phase, a stable oil-in-water microemulsion is formed in which component (a) is in solution in the micro dispersed oil particles, the said preconcentrate being free from a hydrophilic phase.

The invention also provides a process for making a preconcentrate or microemulsion of the invention, wherein component (a) is dissolved directly in component (b) and not in component (d). It is to be understood that component (a) is

dissolved directly in component (b) rather than first being dissolved in another liquid and the solution then mixed with component (b).

EP-A-327280 describes dissolving cyclosporin in a mono- or di-glyceride of a C_6 - C_{10} fatty acid. The solution can then be emulsified in an aqueous medium. However, these emulsions are not microemulsions and do not contain the mixture of lecithin and another surfactant which is especially used, together with the particular triglycerides component (b) all of which are necessary to obtain the significant advantages of the invention.

Microemulsions are transparent due to the very small particle size of the dispersed phase, typically less than 200nm. Such small droplets produce only weak scattering of visible light when compared with that from the coarse droplets (1-10 m) of normal emulsions. An essential difference between microemulsions and emulsions is that microemulsions form spontaneously and, unlike emulsions, require little mechanical work in their formulation. General reviews on microemulsions are provided by Attwood, D. et al, J. Colloid Interface Sci. 46:249 and Kahlweit, M. et al, J. Colloid Interface Sci. 118:436.

In the present invention, component (a) is a water-insoluble pharmaceutically active material. The invention is particularly useful with the cyclosporins, e.g. cyclosporin A, dihydrocyclosporin C, cyclosporin D and dihydrocyclosporin D. It is also useful with other water-insoluble substances such as, for example, taxol.

In the compositions of the invention, component (a) is in solution in component (b). Component (b) can be a single glyceride or any mixture of glycerides (mono- and/or di- and/or tri-) derived from vegetable oils and containing C_8 to C_{20} fatty acid residues. The preferred oils are coconut oil, peanut oil and castor oil. The whole oils can be used or the refined glycerides. The preferred glycerides are those containing C_{12} to C_{18} fatty acid residues, especially triglycerides, and these are the major components of the preferred oils.

The compositions of the three oils are as follows:

Castor Oil:

Tryglycerides of:	ricinoleic acid	87%
	oleic acid	7%
	linoleic acid	3%
	palmitic acid	2%
	stearic acid	1%

and dihydroxystearic acid in trace amounts

Coconut oil:

Tryglycerides of mainly lauric and myristic acids with smaller proportions of capric, caproic acid, caprylic acid, oleic acid, palmitic acid and stearic acid.

Peanut oil:

Glycerides of:	oleic acid	56%
	linoleic acid	26%
	palmitic acid	8.3%
	stearic acid	3.1%
	arachidic acid	2.4%
	behenic acid	3.1%
	lignoceric acid	1.1%

and capric and lauric acid in trace amounts.

Component (c) is a mixture of a phospholipid, preferably lecithin, and another surfactant to provide the stable microemulsion. Those skilled in the art will be aware of many surfactants which can be used, but we prefer to use polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate. These surfactants are extremely effective with lecithin and are highly preferred. Any lecithin can be used but we prefer soya lecithin and egg lecithin. Hydroxylated lecithins are particularly suitable, especially when component (a) is a clyclosporin, since lecithin per se can be lipophilic to an extent sufficient to affect the desired spontaneous formation of a microemulsion.

In the microemulsions of the invention, component (d) is a hydrophilic phase. The preferred material is propylene glycol, but other substances can be used. Ethanol cannot be present. Water can of course also be present but it is not preferred. Despite the use of propylene glycol, component (a) remains wholly dissolved in the oil phase (component (b)).

In use, the microemulsion preconcentrates of the invention are diluted with aqueous liquid (eg. water, fruit juice, milk, etc) to form an oil-in-water microemulsion, e.g. for oral administration. This aids in ready absorption as the surface area of the globules is largely increased. The role played by bile salts in the initial step of fragmentation of fat globules, essential for fat digestion, is circumvented.

In the compositions of the invention, the polar phospholipid lecithin aids in emulsification of the fat and absorption of triglycerides into the GIT. The combination of HLB values of the polar lecithin and for example, the polyoxyl-40-hydrogenated castor oil, is very suitable for forming a balanced microemulsion.

The rate determining factor for the absorption of drug in the vehicle is not the enzymatic metabolism of triglycerides but rests primarily in the breakdown of the fat globules into micro particles since the enzymes (lipases) act mainly at the surface of the fat globules.

In the preconcentrates of the invention, the amounts of the components, in percent by weight, are as follows:

Component		General	Usual	Preferred
(a)	active pharmaceutical	1-12%	2.5-10%	7-10%
(b)	oil phase	20-80%	30-60%	40-50%
(c)	phospholipid	1-10%	3-8%	5-6%
	other surfactant	10-60%	20-50%	35-40%

In the microemulsions, the weight percent of hydrophilic phase is generally up to about 75%, most usually from 15 to 50%, and preferably from 35 to 50%.

The compositions can consist only of the components described, or they can contain other substances. For example, in order to prevent oxidation/rancidification of the natural oils, an antioxidant, e.g. α-tocopherol can be used. Propyl gallate may be used as an alternative.

In order that the invention may be more fully understood, the following Examples are given by way of illustration only.

EXAMPLES 1-4

Microemulsion preconcentrates were made of the substances indicated, by simple mixing. The cyclosporin A was completely dissolved in the oil phase in each case.

Preconcentrate 1:	
Component	Parts
Castor oil	3.0700
Coconut oil	1.6050
Polyoxyl-40 Hydrogenated Castor oil	3.7500
Lecithin	0.5650
α- tocopherol	0.0100
Cyclosporin A	1.0000

Preconcentrate 2:	
Component	Parts
Castor oil	3.1450
Arachis oil	1.5425
Polysorbate-80 (Tween 80)	3.7500
Lecithin	0.5525
α- tocopherol	0.0100
Cyclosporine A	1.0000

Preconcentrate 3:	
Component	Parts
Castor oil	4.1484
Coconut oil	2.0416
Polyoxyl-40 Hydrogenated Castor oil	2.5000
Lecithin	0.3000
α- tocopherol	0.0100
Cyclosporine A	1.0000

Preconcentrate 4:	
Component	Parts
Castor oil	4.690
Coconut oil	1.500
Polysorbate-80 (Tween 80)	2.500
Lecithin	0.300
α- tocopherol	0.010
Cyclosporin A	1.000

When diluted with water or propylene glycol, or another hydrophilic substance, oil-in-water microemulsions formed spontaneously. There was no evidence of any insolubilisation of the cyclosporin.

The microemulsion preconcentrates were filled into bottles to be administered as a drink solution using a syringe or more preferably with the aid of a metered dose pump with a droper actuator. The formulations were also encapsulated in soft gelatin capsules.

The compositions described in Examples 1 to 4 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C), Ref, 40°C-80% RH and 45°C after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Sandimmun Neoral capsules containing 100 mg cyclosporin A per capsule was also examined.

The quantative determination of cyclosporin A was performed by using HPLC method under the following conditions of chromatography:

Pump	: Waters -510 HPLC Pump
Detector	: Waters -484 tunable absorbance detector
Injector	: Waters -715 ultra wisp sample processor
Column	: 4.6 mm x 25 cm column with L16 packing
Thermostat	: 70° - For capsules
	50° - For oral solution
Eluant	: Filtered and degassed mixture of acetronitrile, water, methanol and phosphoric acid (550:400:50:0.5)
Flow rate	: 1 ml/min of the eluant
Integrator	: Waters -746

It was observed from the above examinations that the stability of solutions prepared according to the process of the invention did not differ from the stability of the commercially available composition.

Examples 5-9

Microemulsions of the invention were made of the compositions indicated, by dissolving the cyclosporin A in the oils and then forming the oil-in-water emulsions. The procedure was:

(a) dissolve the cyclosporin A in the mixture of oils with slight warming and under stirring to obtain a clear yellow liquid. Confirm the complete dissolution of the drug by microscopy.
(b) add the surfactant and hydroxylated lecithin in that order, with stirring.
(c) add the propylene glycol with stirring. Stirring was continued for an hour to ensure the formation of a homogeneous translucent to opalescent microemulsion.
(d) add the alpha tocopherol and mix thoroughly.

Example 5:

Preparation of W/O microemulsion for administration in Soft Gelatin capsules:

Component	Parts
Castor oil	1.7200
Coconut oil	0.8000
Polyoxyl-40 Hydrogenated Castor oil	3.3512
Lecithin	0.4200
α- tocopherol	0.0088
Propylene glycol	1.5000
Cyclosporin A	1.0000

Example 6:

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor Oil	1.2700
Arachis oil	0.6050
Polysorbate-80 (Tween 80)	3.7500
Lecithin	0.5525
α- tocopherol	2.0100
Propylene glycol	2.8125
Cyclosporin A	1.0000

Example 7:

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor oil	1.3550
Coconut oil	0.6450
Polyoxyl-40 Hydrogenated Castor oil	3.7500
Lecithin	0.5525
α- tocopherol	0.0100
Propylene glycol	2.6875
Cyclosporin A	1.0000

Example 8

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor oil	0.800
Coconut oil	0.200
Polysorbate-80 (Tween 80)	2.490
Lecithin	0.300
α- tocopherol	0.010
Propylene glycol	5.200
Cyclosporin A	1.000

Example 9

Preparation of O/W microemulsion for administration as oral solution:

Component	Parts
Castor oil	1.200
Coconut oil	0.300
Polyoxyl-40 Hydrogenated Castor oil	2.490
Lecithin	0.300
α- tocopherol	0.010
Propylene glycol	4.700
Cyclosporin A	1.000

The oral solution which is filled into bottles can be administered using a syringe or more preferably with the aid of a metered dose pump with a dropper actuator.

The compositions described in Examples 5 to 9 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C), Ref, 40°C-80% RH and 45°C after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Sandimmun Neoral capsules containing 100 mg cyclosporin A per capsule was also examined.

The quantitative determination of cyclosporin A was performed by using HPLC method under the conditions previously noted (Examples 1 to 4).

It was observed from the above examination that the stability of solutions prepared according to the process of invention did not differ from the stability of the commercially available composition.

Example 10

A drink formulation was made by taking an appropriate amount of the preconcentrate of Example 1 (to give the prescribed dose of cyclosporin A) and adding about 50 ml (or a glassful) of orange-flavoured cordial. The mixture was stirred and was then ready for oral consumption.

Claims

1. A pharmaceutical composition in the form of a preconcentrate for mixture with a hydrophilic phase to form a micro-emulsion, which composition comprises:

   a) a water-insoluble pharmaceutically active material;
   b) C_8 to C_{20} fatty acid mono-, di- or tri-glycerides from a vegetable oil or any mixture of two or more thereof; and
   c) a phospholipid and another surfactant;

   wherein component (a) is directly dissolved in component (b), and component (c) is such that, upon mixing the composition with a hydrophilic phase, a stable oil-in-water microemulsion is formed in which component (a) is in solution in the micro dispersed oil particles, the said preconcentrate being free from a hydrophilic phase.

2. A pharmaceutical composition in the form of a stable oil-in-water microemulsion, which composition comprises

   a) a water-insoluble pharmaceutically active material;
   b) C_8 to C_{20} fatty acid mono-, di-, or tri-glycerides from a vegetable oil, or any mixture of two or more thereof;
   c) a phospholipid and another surfactant; and
   d) a hydrophilic phase;

   wherein component (a) has been wholly directly dissolved in component (b), component (b) is dispersed as tiny particles in component (d), and the composition is free from ethanol.

3. A composition according to claim 1 or 2, wherein component (a) is a cyclosporin, or another water-insoluble peptide, or a water-insoluble antimicrobial or antineoplastic substance.

4. A composition according to claim 3, wherein component (a) is cyclosporin A, dihydrocyclosporin C, cyclosporin D or dihydrocyclosporin D, or desmopresin, calcitonin, insulin, leuprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol or etoposide.

5. A composition according to claim 1,2,3 or 4, wherein in component (b) the glycerides are of C_{12} to C_{18} fatty acids.

6. A composition according to claim 1,2,3,4 or 5, wherein component (b) is whole castor oil, peanut oil or coconut oil, or is derived therefrom.

7. A composition according to any of claims 1 to 6, wherein the phospholipid in component (c) is lecithin, preferably soya lecithin or egg lecithin.

8. A composition according to claim 7, wherein in component (c) the lecithin is hydroxylated lecithin.

9. A composition according to any of claims 1 to 8, wherein in component (c) said surfactant is polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate.

10. A composition according to any of claims 1 to 9, wherein the weight ratio of component (a) to component (b) is from 1:1 to 1:10.

11. A composition according to any of claims 1 to 10, wherein the weight ratio of component (a) to said phospholipid is from 1:0.5 to 1:5.0.

12. A composition according to any of claims 1 to 11, wherein the weight ratio of component (a) to said surfactant is from 1:1 to 1:5.0.

13. A process for making a composition according to claim 2, which comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution with component (d) and component (c) if not included earlier.

14. A process according to claim 13, wherein a preconcentrate composition as claimed in claim 1 is mixed with component (d).

15. A soft gelatin capsule which comprises a composition as claimed in claim 2, or as claimed in any of claims 3 to 12 when dependent on claim 2.

16. An oral administration fluid which comprises a composition as claimed in claim 2, or as claimed in any of claims 3 to 12 when dependent on claim 2.

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EUROPEAN SEARCH REPORT

Application Number
EP 95 30 6022

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	DE-A-32 25 706 (A.NATTERMANN & CIE GMBH) * claims 1-13 * * page 7, line 13 - line 17 * ---	1,3,5,7, 13,14	A61K9/107 A61K38/13
X	WO-A-93 18752 (PHARMOS CORP.) * claims 1-15,22-24 * * page 8, line 10 - page 9, line 35 * * page 12, line 16 - line 26 * ---	2-9,13	
X	EP-A-0 521 799 (YISSUM RESEARCH DEVELOPMENT COMPANY.....) * claims 1-10 * * page 3, line 30 - line 41 * * page 4, line 43 - page 5, line 3 * ---	2,5-8, 13,14	
X	EP-A-0 429 248 (SHISEIDO COMPANY LIMITED) * claims 1-10 * ---	2-9,13, 14	
Y	EP-A-0 651 995 (DR. HANS DIETL) * claims 1-20 * * page 5, line 20 - line 23 * * example 1 * ---	2-11, 13-16	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K
Y,D	EP-A-0 327 280 (SANKYO COMPANY LTD) * claims 1-22 * * page 8; examples 1-4 * ---	2-11, 13-16	
A,D	EP-A-0 589 843 (SANDOZ AG) * claims 1-10 * * page 6, line 2 - line 7 * ---	1-16	
A	FR-A-2 636 534 (SANDOZ S.A.) * claims 1-30 *	1-16	
D	& GB-A-2 222 770 -----		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	7 March 1996	Siatou, E

CATEGORY OF CITED DOCUMENTS

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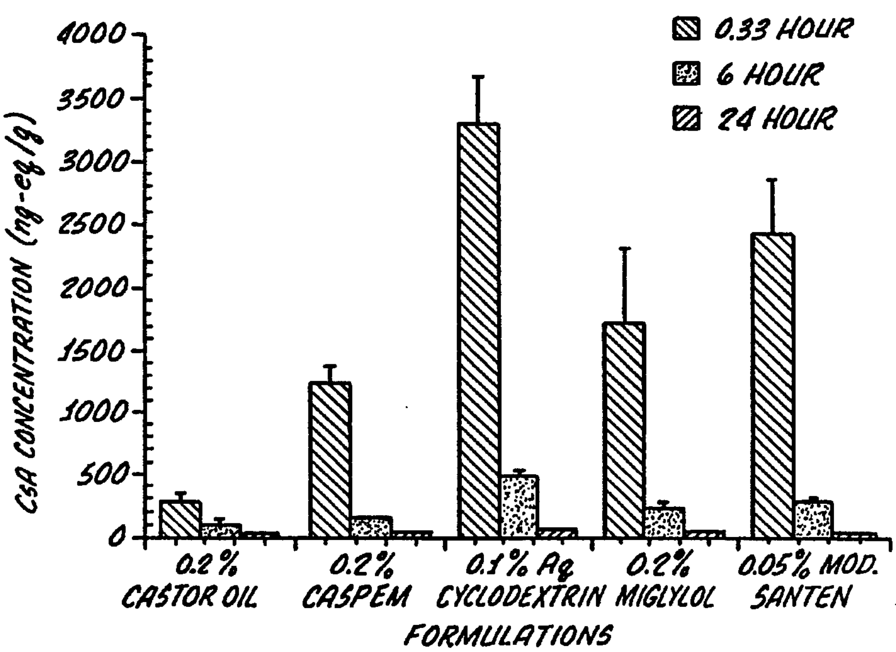

(57) Abstract

A pharmaceutical composition is disclosed in the form of a nonirrtating emulsion which includes at least one cyclosporin in admixture with a higher fatty acid glyceride and polysorbate 80. More particularly, the cyclosporin may be cyclosporing A and the higher fatty acid glyceride may be castor oil. Composition has been found to be of a high comfort level and low irritation potential suitable for delivery of medications to sensitive areas such as ocular tissues with enhanced absorption in the lacrimal gland. In addition, the composition has stability for up to nine months without crystallization of cyclosporin.

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LACRIMAL GLAND SPECIFIC EMULSIONS FOR TOPICAL APPLICATION TO OCULAR TISSUE

This application is a continuation-in-part of pending US patent application SN 08/243,279 filed May 17, 1994.

The present invention generally relates to novel pharmaceutical compositions incorporating chemicals which are poorly soluble in water and is more particularly related to a novel ophthalmic emulsion including cyclosporin in admixture with castor oil and polysorbate 80 with high comfort level and low irritation potential.

Cyclosporins are a group of nonpolar cyclic oligopeptides with known immunosuppressant activity. In addition, as set forth in U.S. Patent No. 4,839,342, cyclosporin (sometimes referred to in the literature as "cyclosporine") has been found as effective in treating immune medicated keratoconjunctivitis sicca (KCS or dry eye disease) in a patient suffering therefrom.

As hereinabove noted, cyclosporin comprises a group of cyclic oligopeptides and the major component thereof is cyclosporin A ($C_{62}H_{111}N_{11}O_{12}$)which has been identified along with several other minor metabolites, cyclosporin B through I. In addition, a number of synthetic analogs have been prepared.

In general, commercially available cyclosporins may contain a mixture of several individual cyclosporins which all share a cyclic peptide structure consisting of eleven amino acid residues with a total molecular weight of about 1,200, but with different

substituents or configurations of some of the amino acids.

It should be appreciated that reference to the term "cyclosporin" or "cyclosporins" is used throughout the present specification in order to designate the cyclosporin component in the composition of the present invention.

However, this specific reference is intended to include any individual member of the cyclosporin group as well as admixtures of two or more individual cyclosporins, whether natural or synthetic.

The activity of cyclosporins, as hereinabove noted, is as an immunosuppressant and in the enhancement or restoring of lacrimal gland tearing.

This activity can be enhanced if it is possible to enhance the absorption of the cyclosporin in the lacrimal gland. The present invention provides for a formulation and method that produces optimal cyclosporin A concentrations in the lacrimal gland and other ocular surface tissues.

Unfortunately, the solubility of cyclosporin in water is extremely low and as elaborated in U.S. Patent No. 5,051,402, it has been considered not merely difficult but practically impossible to prepare a pharmaceutical composition containing cyclosporin dissolved in an aqueous medium.

As reported, the solubility of cyclosporin in water is between about 20 μg/ml to 30 μg/ml for cyclosporin A. Hence, heretofore prepared formulations incorporating cyclosporin have been prepared as oily solutions containing ethanol. However, these

preparations limit the bioavailability to oral preparations and this is believed to be due to the separation of cyclosporin as a solid immediately after it comes into contact with water, such as in the mouth or eye of a patient.

In the case of injectable preparations of cyclosporin, they first must be diluted with physiological saline before intravenous administration but this is likely to result in the precipitation of cyclosporin and therefore may be considered undesirable for intravenous administration.

Surface active agents such as polyoxyethylated castor oil have been utilized as solubilizers to inject preparations in order to prevent cyclosporin from separating. However, this also may give rise to safety problems (see U.S. Patent No. 5,051,402).

The practical usefulness of cyclosporin would be greatly enhanced if administration thereof could be effective; for example, cyclosporin's effectiveness in the treatment of ocular symptoms of Behcet's Syndrome. However, if it is administered orally for the treatment of these symptoms, the accompanying side effects due to systemic circulation may cause adverse reactions such as hypertrichosis or renal dysfunction.

On the other hand, if oily preparations containing cyclosporin are applied directly to the eyes, irritation or a clouding of visual field may result. This plus the difficulty in formulating cyclosporin limits its use in formulations that would be useful during keratoplasty as well in the treatment of herpetic keratitis and spring catarrh.

Heretofore, as for example in U.S. Patent No. 5,051,402, attempts have been made to dissolve sufficient cyclosporin in an aqueous solvent system so as to reach an effective concentration for treatment. Importantly, this solvent system does not contain any surface active agent such as polyoxyethylated castor oil.

Conceptually, the purpose of dissolving the cyclosporin in an aqueous solvent system is to enable contact with body fluids which would merely constitute dilution of the aqueous solvent system which hopefully would eliminate the immediate precipitation of cyclosporin when contacted with the water content of the body fluids.

For direct use in the eye, cyclosporin has been formulated with a number of pharmaceutically acceptable excipients, for example, animal oil, vegetable oil, an appropriate organic or aqueous solvent, an artificial tear solution, a natural or synthetic polymer or an appropriate membrane.

Specific examples of these pharmaceutically acceptable excipients, which may be used solely or in combination, are olive oil, arachis oil, castor oil, mineral oil, petroleum jelly, dimethyl sulfoxide, chremophor, liposomes, or liposome-like products or a silicone fluid, among others.

In summary, a great deal of effort has been expended in order to prepare a pharmaceutical composition containing cyclosporin dissolved in an aqueous medium or cyclosporin prepared as an oily solution. However, successful formulations have yet to be accomplished as evidenced by the lack of commercial products.

As hereinabove noted, it has been reported that cyclosporin has demonstrated some solubility in oily preparations containing higher fatty acid glycerides such as olive oil, peanut oil, and/or castor oil. These formulations frequently produce an unpleasant sensation when applied to the eye because of stimulation or the viscousness which is characteristic of these oils.

Another drawback of these formulations is that they contain a high concentration of oils, and oils exacerbate the symptoms of certain ocular surface diseases such as dry eyes, indicated by cyclosporin. Therefore, these oily formulations may not be clinically acceptable. Additionally, these formulations often suffer from physical instability due to cyclosporin's propensity to undergo conformational change and crystallize out. The crystallization problem has been noticed in formulations containing corn oil or medium chain triglycerides. Lastly, these formulations often have a low thermodynamic activity (degree of saturation) of cyclosporin which leads to a poorer drug bioavailability.

It may be possible to minimize the problems related to unpleasant sensation and syndrome exacerbation by reducing the oil content and dispersing the oil phase in water into an emulsion. However, it is not an easy task to formulate an ophthalmic emulsion because one indispensable class of ingredients in an emulsion system is emulsifiers, and the majority of emulsifiers is highly irritating to the eyes.

The present invention is directed to an emulsion system which utilizes higher fatty acid glycerides but in combination with polysorbate 80 which results in an emulsion with a high comfort level and low irritation

potential suitable for delivery of medications to sensitive areas such as ocular tissues. Further, the present invention provides a pharmaceutical composition and method for causing preferential absorption of cyclosporin in the lacrimal gland. That is, for a given instillation of the composition into an eye, a greater amount of absorption occurs in the lacrimal gland for formulations made in accordance with the present invention than heretofore utilized formulations.

SUMMARY OF THE INVENTION

In accordance with the present invention, a non-irritating pharmaceutical composition with high comfort level and low irritation potential suitable for delivery to sensitive areas such as ocular tissues comprises cyclosporin in admixture with an emulsifying amount of a higher fatty acid glycerol and polysorbate 80. More particularly, the composition may comprise cyclosporin A and the higher fatty acid glyceride may comprise castor oil.

Preferably, the weight ratio of the castor oil to the polysorbate 80 is between about 0.3 to about 30 and a weight ratio of the cyclosporin to castor oil is below 0.16. More preferably, the weight ratio of castor oil to polysorbate 80 is between 0.5 and 12.5, and the weight ratio of cyclosporin to castor oil is between 0.12 and 0.02.

When cyclosporin is dissolved in the oil phase in accordance with the present invention, the emulsion is found to be physically stable upon long term storage. No crystallization of cyclosporin was noticed after nine months at room temperature. Moreover, the cyclosporin emulsion is formulated in such a way that

the drug has reasonably high thermodynamic activity, yet without the crystallization problem.

Importantly, the composition of the present invention provides for enhanced absorption of the cyclosporin in the lacrimal gland of the eye. In this manner, the activity of the cyclosporin in restoring lacrimal gland tearing is increased. That is, since a greater amount of cyclosporin is absorbed into the lacrimal gland, more of the cyclosporin is effective in producing lacrimal gland tearing than heretofore possible.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantages and features of the present invention will be better understood by the following description when considered in conjunction with the accompanying drawings in which:

Figure 1 is a bar chart of conjunctival concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye;

Figure 2 is a bar chart of cornea concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye;

Figure 3 is a bar chart of ciliary body concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye; and

Figure 4 is a bar chart of lacrimal gland concentration of cyclosporin A after a single topical instillation of various formulations in a rabbit eye.

DETAILED DESCRIPTION

As hereinabove noted, cyclosporin is available as a mixture in which the principal ingredient is cyclo-

sporin A with significant, but smaller, quantities of other cyclosporins such as cyclosporin B through I. However, as also hereinabove noted, the present invention may be applied to either a pure cyclosporin or to a mixture of individual cyclosporins.

The discovery on which the present invention is founded relates to a combination of a higher fatty acid glyceride and an emulsifier and dispersing agent, polysorbate 80. The selection of these components could not have been anticipated on the basis of conventional thinking.

For example, although it is well known that cyclosporin may be used in combination with castor oil, this combination is irritating to sensitive tissues such as the eye. Thus, conventional teaching in the art is away from a formulation which utilizes a higher fatty acid glyceride, such as castor oil, and cyclosporin.

Stated another way, there is no way of deducing that the use of an emulsifier and dispersing agent such as polysorbate 80 will reduce the irritation potential of an emulsion utilizing castor oil. There are no examples of polysorbate in combination with castor oil which, when admixed to cyclosporin, produces an emulsion with a high comfort level and low irritation potential suitable for the delivery of medication to sensitive areas such as ocular tissues.

The present invention achieves a <u>stable solution state</u> of cyclosporin. This stable solution state is another important performance characteristic differentiating the present invention from the conventional oil systems. Cyclosporin is notorious for its ten-

dency to precipitate out in conventional oil systems in which it is fully dissolved initially.

In accordance with the present invention, the emulsions can be further stabilized using a polyelectrolyte, or polyelectrolytes if more than one, from the family of cross-linked polyacrylates, such as carbomers and Pemulen®.

Pemulen® is a polymeric emulsifier having a CTFA name of Acrylates/C10-30 Alkyl Acrylate Cross-Polymer and is discrived in th "Carbomer 1342" monograph in the USPXXII/NFXVII.

In addition, the tonicity of the emulsions can be further adjusted using glycerine, mannitol, or sorbitol if desired. The Ph of the emulsions can be adjusted in a conventional manner using sodium hydroxide to a near physiological pH level and while buffering agents are not required, suitable buffers may include phosphates, citrates, acetates and borates.

While the preferable medications in accordance with the present invention include cyclosporin, other chemicals which are poorly soluble in water such as indomethacin and steroids such as androgens, prednisolone, prednisolone acetate, fluorometholone, and dexamethasones, may be emulsified with castor oil and polysorbate 80 resulting in a composition with similar low irritation potential.

The invention is further illustrated by the following examples with all parts and percentages expressed by weight. The cyclosporin used in the examples was supplied by Sandoz.

Example 1

	A	B	C	D	E
Cyclosporin A	0.40%	0.20%	0.20%	0.10%	0.05%
Castor oil	5.00%	5.00%	2.50%	1.25%	0.625%
Polysorbate 80	1.00%	1.00%	1.00%	1.00%	1.00%
Pemulen®	0.05%	0.05%	0.05%	0.05%	0.05%
Glycerine	2.20%	2.20%	2.20%	2.20%	2.20%
NaOH	qs	qs	qs	qs	qs
Purified water	qs	qs	qs	qs	qs
pH	7.2-7.6	7.2-7.6	7.2-7.6	7.2-7.6	7.2-7.6

Example 2

	A	B	C	D
Castor oil	5.00%	2.50%	1.25%	0.625%
Polysorbate 80	1.00%	1.00%	1.00%	1.00%
Pemulen®	0.05%	0.05%	0.05%	0.05%
Glycerine	2.20%	2.20%	2.20%	2.20%
NaOH	qs	qs	qs	qs
Purified water	qs	qs	qs	qs
pH	7.2-7.6	7.2-7.6	7.2-7.6	7.2-7.6

Example 3

	A
Castor oil	2.50%
Polysorbate 80	0.75%
Carbomer 1382	0.05%
Glycerine	2.20%
NaOH	qs
Purified water	qs
pH	7.2-7.6

Example 4

	A
Castor oil	5.00%
Polysorbate 80	0.75%
Carbomer 981	0.05%
Glycerine	2.20%
NaOH	qs
Purified water	qs
pH	7.2-7.6

The formulations set forth in Examples 1-4 were made for treatment of keratoconjunctivitis sicca (dry eye) syndrome with Examples 2, 3 and 4 without the active ingredient cyclosporin utilized to determine the toxicity of the emulsified components.

The formulations in Examples 1-4 were applied to rabbit eyes eight times a day for seven days and were found to cause only slight to mild discomfort and slight hyperemia in the rabbit eyes. Slit lamp examination revealed no changes in the surface tissue. In addition, the cyclosporin containing castor oil emulsion, as hereinabove set forth in Examples 1A-1D, was also tested for ocular bioavailability in rabbits; and the therapeutic level of cyclosporin was found in the tissues of interest after dosage. This substantiates that cyclosporin in an ophthalmic delivery system is useful for treating dry eye as set forth in U.S. Patent No. 4,839,342.

In addition, no difference in toxicity was found between formulations with cyclosporin (Examples 1A-1D) and formulations without cyclosporin (Examples 2-4).

The formulations set forth in Examples 1-4 were found to be physically stable upon long term storage.

With regard to formulations 1A-1D, no crystallization of cyclosporin was noticed after nine months at room temperature.

Further, other higher fatty acid glycerides such as olive oil, peanut oil and the like may also be utilized with the polysorbate 80 with similar results regarding biotoxicity.

The following examples demonstrate the activity of the composition in accordance with the present invention for enhanced absorption of cyclosporin A in the lacrimal gland.

Materials

The [Mebmt-^{3}H]-cyclosporin-A (lot #TRQ6553) was prepared by Amersham International (Buckinghamshire, England) with radiochemical purity of ~98% (by reversed phase HPLC) and specific activity of 2.6 Ci/mmol (2.16 mCi/mg). The ^{3}H-label is a metabolically stable position as shown by the asterisk. The radiolabeled CsA was supplied as an ethanol solution (1 mCi/ml). All organic solvents used in the procedures described in this study were "HPLC grade". all other chemicals and reagents were analytical grade unless otherwise noted.

The compositions of the six formulations tested are listed in Table A.

TABLE A

Ingredients	Castor Oil	Castor Oil-in-Water Emulsion	Aqueous-α Cyclo-dextrin	Miglyol Oil-in-Water Emulsion	Polyoxyl 40	Polyoxyl 40 with Edetate
Cyclosporin-A	0.20	0.20	0.10	0.20	0.05	0.05
Cyclodextrin			14			
Castor Oil	99.8	1.25				
Miglyol Oil				20		
Pluronic L121+P123				0.75		
Tween 80		1.00				
Glycerin		2.20		2.20		
Pemulen® TR-2		0.05				
Carbopol 981				0.05		
Polyoxyl 40 Stearate (mg)					20	20
HPMC					0.3	0.3
Butylated Hydroxytoluene					0.001	0.001
Ethanol(9200 proof)						0.1
Sodium Chloride					0.73	0.73
Sodium Monophosphate					0.2	0.2
Disodium Edetate						0.1
Water		QS	QS	QS	QS	QS
Batch Size	1 g	5 g	1 g	5 g	1 g	1 g

The radiolabeled formulations were formulated to ensure that the radioactivity was homogeneous throughout the vehicle. The expected radioactivity concentrations of the radiolabeled drug formulations were 1-2 mCi/ml. The expected specific activity of radiolabeled cyclosporin A (CsA) formulations was 0.5-2 mCi/mg. All test articles were stored at ambient temperature.

Analysis of Test Drug Formulations

The test formulations were analyzed in triplicate by HPLC to determine the concentration of CsA and radiochemical purity of the CsA dosing solutions (>93%) before dosing. The radioactive concentrations of the test formulations were quantified by liquid scintillation counting (LSC).

Chromatographic Conditions

Pump:	Beckman Model 126 (Beckman Instruments, San Ramon, CA)
Mobile phase:	Acetonitrile: 0.03% H_3PO_4 in water, pH 3 (65:35 v/v)
Flow rate:	1.0 ml/min
Column:	Supercosil C8, 7.5 cm x 4.6 mm, 3 μm (Supelco, Bellefonte, PA) Superguard LC-8 (Supelco) Column heater (Bio-rad, Richmond, CA) at 60-70°C
Injector:	WISP 712B (Waters Associates, Milford, MA)
^{14}C detector:	Radio Isotope 171 Detector (Beckman Instruments)
Scintillant:	Ready Flow III (Beckman Instruments), Flow Rate of ˜4 ml/min
UV detector:	Model 166 (Beckman Instruments), 202 nm
Data processor:	Beckman System Gold (Beckman Instruments)
Run Time:	15 min
Retention Time:	6 min (cyclosporin A)

Animals

Female New Female New Zealand albino rabbits were obtained and quarantined for at least five days before procedures. Animals were housed in temperature- and humidity-controlled rooms. Food and tap water were provided *ad libitum*. Fifty-eight rabbits (2-3 kg) were selected from the colony to minimize bias. They were individually identified by ear tags and appeared to be healthy.

Dosing

The animals were divided into six groups of nine rabbits; each group was treated with one of the six CsA formulations. During dosing, the lower eyelid of each rabbit was gently pulled away from the eye and 35 μl of the formulation were administered in the lower conjunctival cul-de-sac of each eye. After dosing, the upper and lower eyelid were handheld closed for ˜5 seconds and released. The animals were observed visually for any signs of tearing or ocular discomfort.

Sampling

Tissues were collected at 20-min., 6-hr. and 24-hr. post-dose for each group. Three rabbits (six eyes) were used at each time point. At the specific sampling times, the animals were euthanized by an intravenous injection of 0.5-1 ml Eutha-6 (Western Supply Co., Arcadia, California) via marginal ear vein. Each eye was then rinsed with normal saline. The aqueous humor (˜200 μl) was removed by means of a 0.5 ml tuberculin syringe. The orbital lacrimal gland (˜400 mg), upper and lower bulbar conjunctivae (˜50 mg each), corneal (˜50 mg) and iris-ciliary body (˜50 mg)

were dissected. The tissues dissected were blotted dry and weighed. Ocular tissue and aqueous humor samples from both eyes were collected from four untreated animals to be used as blank samples.

Analysis of Radioactivity

An aliquot of aqueous humor (50-175 μl) was counted directly in 10 ml of Ready-Solv HP by LSC. Tissue and blood samples were weighed into combustion cones prior to combustion in a Model 307 Packard Tissue Oxuduzer (Packard Co., Downers Grove, Illinois). After combustion of the tissue samples, ^3H_2O was trapped in the Monophase-S solution (Packard) and the radioactivity of the samples was determined by LSC in a Beckman Model 1801 or 3801 scintillation counter (Beckman Instruments, San Ramon, California).

Data Analyses

Excel software (version 4.0, Microsoft Corp, Redmond, Washington) was used for data analysis. concentrations of total radioactivity in the tissue samples were expressed as dpm/g or dpm/ml and converted to ng equivalents (eq) of CsA/g or ml, using the specific activity of the dosing formulations. Mean, standard deviation (SD) or standard error of the mean (SEM) was calculated according to standard methods. Radioactivity levels were not considered significant unless the dpm was greater than twice that of background b=(blanks).

Comparisons of tissue drug concentrations at each time point for the formulations were determined by one-factor ANOVA. All statistical comparisons were made using StatView® (version 1.03, Abacus Concepts, Inc., Berkeley, California). the Fisher and Scheffe

F tests were used to determine significant differences between formulations at the 95% level (α = 0.05). The rejection criteria for excluding any outlier data was based on standard outlier tests. No more than one outlier was eliminated from any data set.

Results and Discussion

The radioactivity concentrations in ocular tissues at 20 minutes, 6 hours, and 24 hours after a single topical application of various formulations are depicted in Figures 1-4. In general, the concentrations in the ocular tissues were greatest at the earliest time point of 20 minutes as reported in previous single dose studies (2, 3). The radioactivity concentration was highest in the conjunctiva and cornea for each formulation. The relatively low aqueous humor and iris-ciliary body concentrations suggest low intraocular absorption of CsA, consistent with the low CsA corneal permeability of -1.0 x 10^{-6} cm/sec (6). The decline of radioactivity concentrations from the cornea was slower than those from the conjunctiva, lacrimal gland, and aqueous humor. The observed blood radioactivity concentrations (<3 ng-eq/ml) were much lower than trough plasma CsA concentrations of 80-250 ng/ml observed after oral dosing to humans (1).

The dependence of CsA corneal and conjunctival penetration on the formulation was interpreted in terms of CsA concentration in formulation and the release rate of CsA from formulation into tear film. The aqueous formulations demonstrated a greater propensity to release CsA for diffusion across the surface tissue epithelia. The 0.2% straight castor oil was formulated below the CsA solubility and therefore the release rate could be hampered by the less than maximal CsA thermodynamic activity (5).

The ocular surface tissues contained a higher fraction of the CsA dose than the other tissues and was used to discriminate among the aqueous, emulsion and the straight castor oil formulations. The polyoxyl 40 formulation produced higher ocular surface tissue concentrations than the emulsions and straight castor oil. The emulsions were also effective in delivery of CsA to the tissues of interest, lacrimal gland, cornea, and conjunctiva. The castor oil emulsion showed higher lacrimal gland concentrations than the modified Santen and the miglyol emulsion. The straight castor oil showed the lowest concentrations in surface ocular tissues. Apparently, the factors influencing CsA penetration into the lacrimal gland and the surface tissues are different.

Although there has been hereinabove described a particular pharmaceutical composition in the form of a nonirritating emulsion for the purpose of illustrating the manner in which the invention may be used to advantage, it should be appreciated that the invention is not limited thereto. Accordingly, any and all modifications, variations, or equivalent arrangements, which may occur to those skilled in the art, should be considered to be within the scope of the present invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a nonirritating emulsion of at least one cyclosporin in admixture with a higher fatty acid glyceride, polysorbate 80 and an emulsion-stabilizing amount of Pemulen® in water suitable for topical application to ocular tissue.

2. The pharmaceutical composition according to claim 1 wherein the cyclosporin comprises cyclosporin A.

3. The pharmaceutical composition according to claim 2 wherein the weight ratio of the higher fatty acid glyceride to the polysorbate 80 is between about 0.3 and about 30.

4. The pharmaceutical composition according to claim 3 wherein the higher fatty acid glyceride comprises castor oil and the weight ratio of cyclosporin to castor oil is below about 0.16.

5. A pharmaceutical composition comprising a nonirritating emulsion of at least one cyclosporin in admixture with castor oil and polysorbate 80 in water suitable for topical application to ocular tissue.

6. The pharmaceutical composition according to claim 5 wherein the cyclosporin comprises cyclosporin A.

7. The pharmaceutical composition according to claim 6 wherein the weight ratio of castor oil to the polysorbate 80 is between about 0.3 and about 30.

8. The pharmaceutical composition according to claim 7 wherein the weight ratio of cyclosporin to castor oil is below about 0.16.

9. The composition according to claim 1 wherein the higher fatty acid glyceride and polysorbate 80 are present in amounts sufficient to prevent crystallization of cyclosporin for a period of up to about nine months.

10. A stable, nonirritating ophthalmic composition comprising cyclosporin in admixture with an emulsifying amount of a higher fatty acid glyceride and polysorbate 80.

11. A pharmaceutical emulsion comprising cyclosporin A, castor oil, Pemulen®, glyceride and water in amounts sufficient to prevent crystallization of cyclosporin A for a period of up to about nine months, said pharmaceutical emulsion being suitable for topical application to ocular tissue.

12. The pharmaceutical emulsion according to claim 11 wherein the cyclosporin A is present in an amount of between about 0.05 to about 0.40%, by weight, the castor oil is present in ana mount of between about 0.625%, by weight, the polysorbate 80 is present in an amount of about 1.0%, by weight, the Pemulen® is present in an amount of about 0.05%, by weight, and the glyceride is present in an amount of about 2.2%, by weight.

13. A pharmaceutical emulsion consisting of between about 0.05% and about 0.40%, by weight, cyclosporin A, between about 0.625% and about 5.0%, by weight, castor oil, about 1.0%, by weight, polysorbate 80, about 0.05%, by weight, Pemulen®, and about 2.2%,

by weight, glycerine in water with a pH of between about 7.2 and 7.6 suitable for topical application to ocular tissue.

14. A pharmaceutical composition suitable for instillation into an eye, said pharmaceutical composition comprising a nonirritating emulsion of at least one cyclosporin and castor oil in an amount causing enhanced lacrimal gland absorption.

15. The pharmaceutical composition according to claim 14 wherein the cyclosporin comprises cyclosporin A.

16. The pharmaceutical composition according to claim 15 wherein the cyclosporin is present in an amount of between about 0.20 and about 5.0% by weight.

17. The pharmaceutical composition according to claim 15 further comprising an emulsion-stabilizing amount of Pemulen® in water suitable for topical application in the eye.

18. The pharmaceutical composition according to claim 17 wherein the cyclosporin is present in an amount of about 0.20% by weight, the castor oil is present in an amount of about 1.25% by weight, and the Pemulen® is present in an amount of about 0.05% by weight.

19. The pharmaceutical composition according to claim 18 further comprising Tween 80 in an amount of about 1.0% by weight, and glycerin in an amount of about 2.20% by weight.

20. A pharmaceutical composition suitable for instillation into an eye, said pharmaceutical

composition comprising a nonirritating admixture of at least one cyclosporin and castor oil in an amount causing enhanced lacrimal gland absorption.

21. The pharmaceutical composition according to claim 20 wherein the cyclosporin comprises cyclosporin A.

22. A method of causing enhanced absorption of cyclosporin A in the lacrimal gland of an eye, said method comprising the steps of:

admixing cyclosporin A with castor oil; and

instilling the admixture into the eye.

23. The method according to claim 22 wherein the step of admixing includes forming an emulsion of cyclosporin A, castor oil and water.

24. A method of causing enhanced absorption of cyclosporin A in the lacrimal gland of an eye, said method comprising the steps of:

forming an emulsion of cyclosporin A, castor oil, Pemulen® and water; and

instilling the emulsion into the eye.

25. The method according to claim 24 wherein the cyclosporin is present in an amount of about 0.20% by weight, the castor oil is present in an amount of about 1.25% by weight, and the Pemulen® is present in an amount of about 0.05% by weight.

26. The method according to claim 24 wherein the emulsion further comprises Tween 80 in an amount of about 1.0% by weight, and glycerin in an amount of about 2.20% by weight.

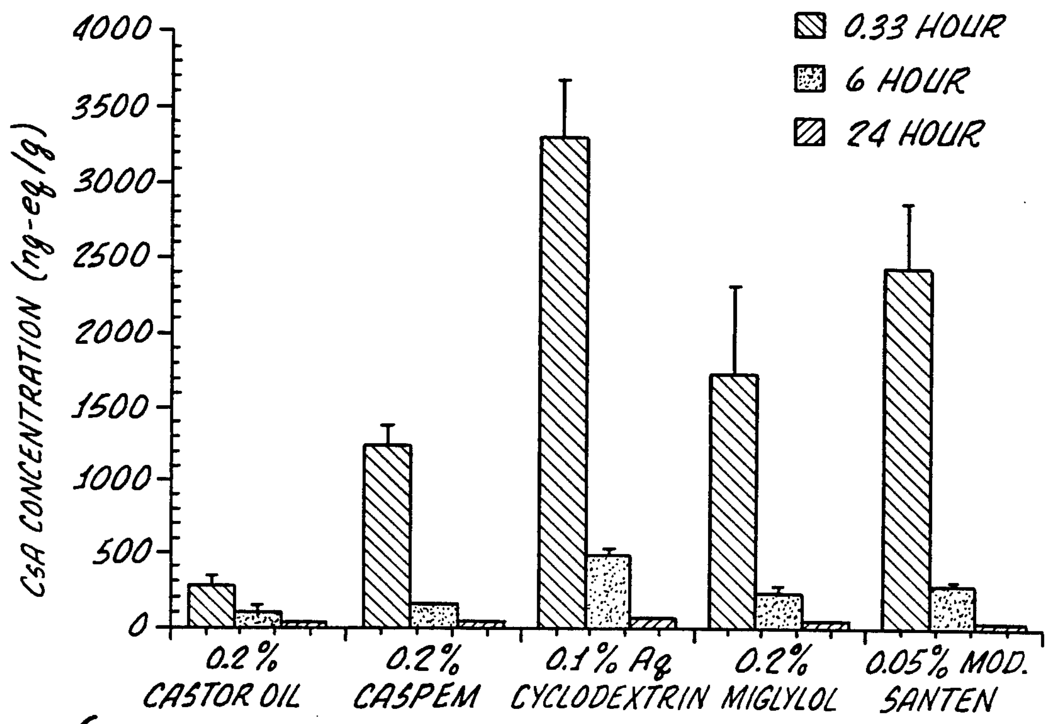
0.33 HOUR
6 HOUR
24 HOUR
CsA CONCENTRATION (ng-eq/g)
4000
3500
3000
2500
2000
1500
1000
500
0
0.2%
CASTOR OIL
0.2%
CASPEM
0.1% Aq
CYCLODEXTRIN
0.2%
MIGLYLOL
0.05% MOD.
SANTEN
FORMULATIONS

FIG. 1.

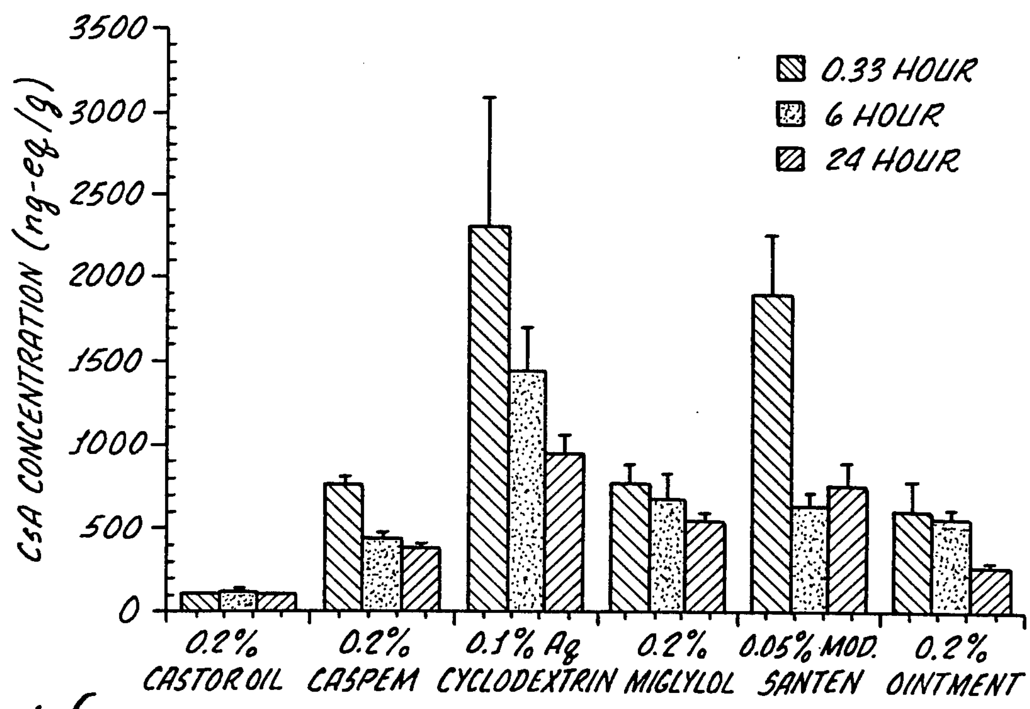
0.33 HOUR
6 HOUR
24 HOUR
CsA CONCENTRATION (ng-eq/g)
3500
3000
2500
2000
1500
1000
500
0
0.2%
CASTOR OIL
0.2%
CASPEM
0.1% Aq
CYCLODEXTRIN
0.2%
MIGLYLOL
0.05% MOD.
SANTEN
0.2%
OINTMENT
FORMULATIONS

FIG. 2.

2/2

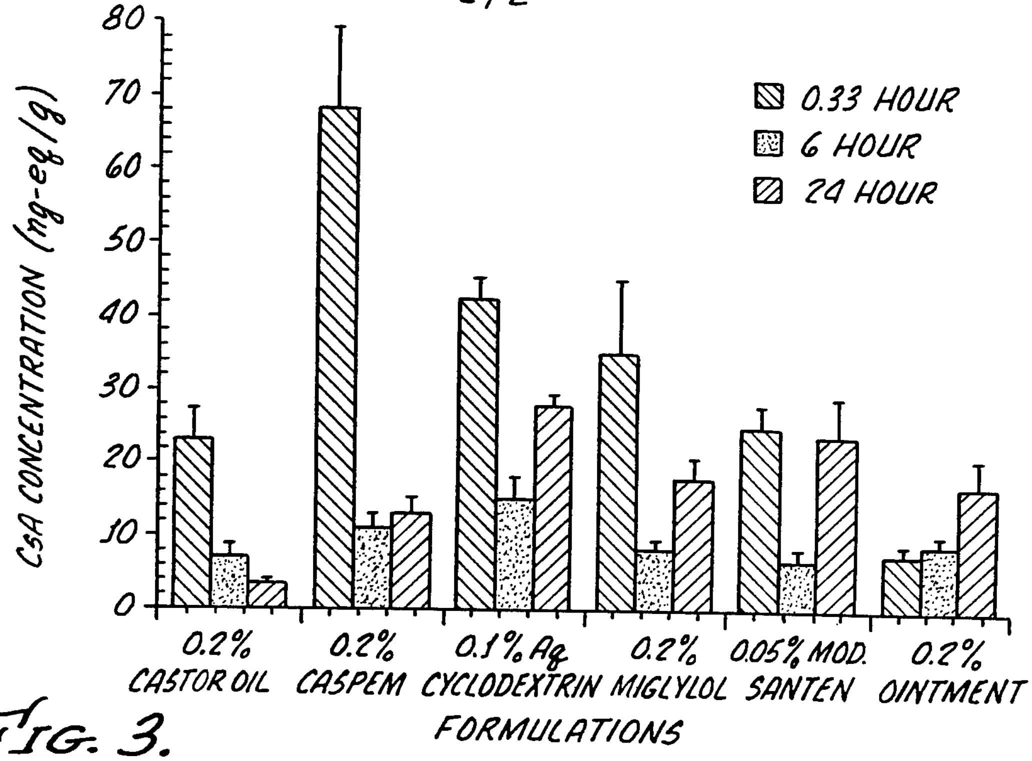
80
70
60
50
40
30
20
10
0
CsA CONCENTRATION (ng-eq/g)
0.33 HOUR
6 HOUR
24 HOUR
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
0.2% OINTMENT
FORMULATIONS

FIG. 3.

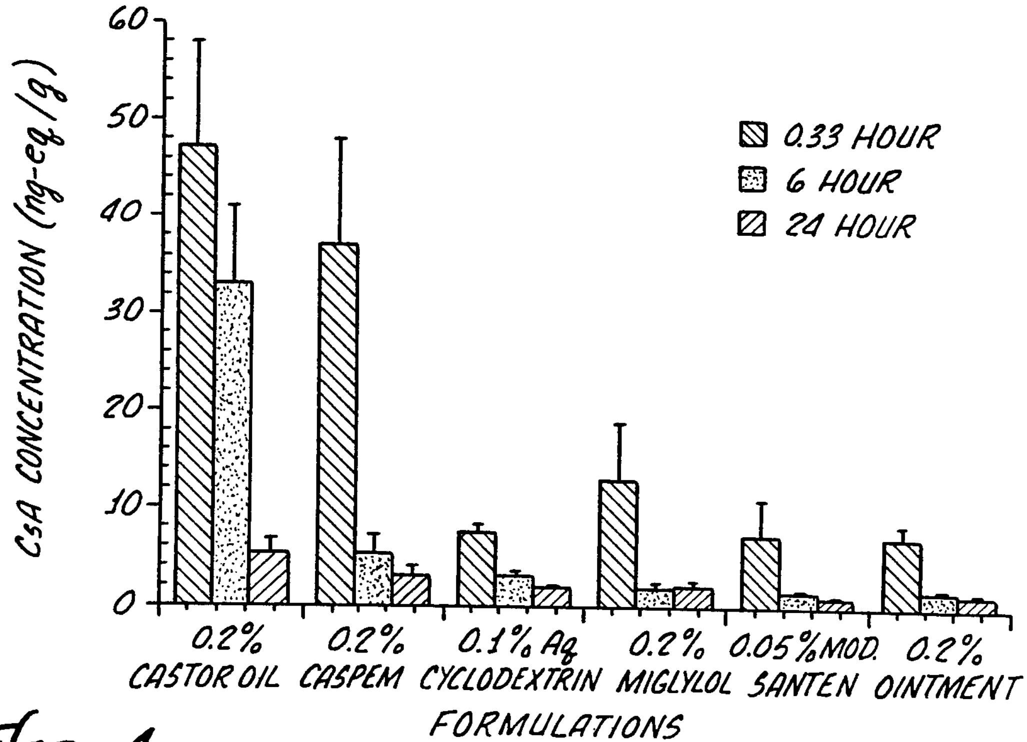
60
50
40
30
20
10
0
CsA CONCENTRATION (ng-eq/g)
0.33 HOUR
6 HOUR
24 HOUR
0.2% CASTOR OIL
0.2% CASPEM
0.1% Aq CYCLODEXTRIN
0.2% MIGLYLOL
0.05% MOD. SANTEN
0.2% OINTMENT
FORMULATIONS

FIG. 4.

INTERNATIONAL SEARCH REPORT

Inten ıal Application No
PCT/US 95/06302

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/13 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 228 198 (SANDOZ LTD.) 22 August 1990	10
Y	see claim 1 see page 13, paragraph 2 see page 27, paragraph 3 ---	5-8
X	WO,A,89 01772 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION INC.) 9 March 1989	14-16, 20,23
Y	see claims 11-13,15 see page 10, line 17 - line 31 see page 11, line 11 - line 17 -----	5-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 1995	26.09.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ventura Amat, A

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern nal Application No
PCT/US 95/06302

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A-2228198	22-08-90	BE-A-	1005236	08-06-93
		CH-A-	680650	15-10-92
		DE-A-	4005190	23-08-90
		FR-A,B	2643262	24-08-90
		IT-B-	1240765	17-12-93
		JP-A-	2255623	16-10-90
		JP-B-	6011703	16-02-94
WO-A-8901772	09-03-89	US-A-	4839342	13-06-89
		AT-T-	109970	15-09-94
		AU-B-	2421388	31-03-89
		DE-D-	3851152	22-09-94
		DE-T-	3851152	26-01-95
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		JP-B-	6067850	31-08-94
		US-A-	5411952	02-05-95
		CA-A-	1335566	16-05-95

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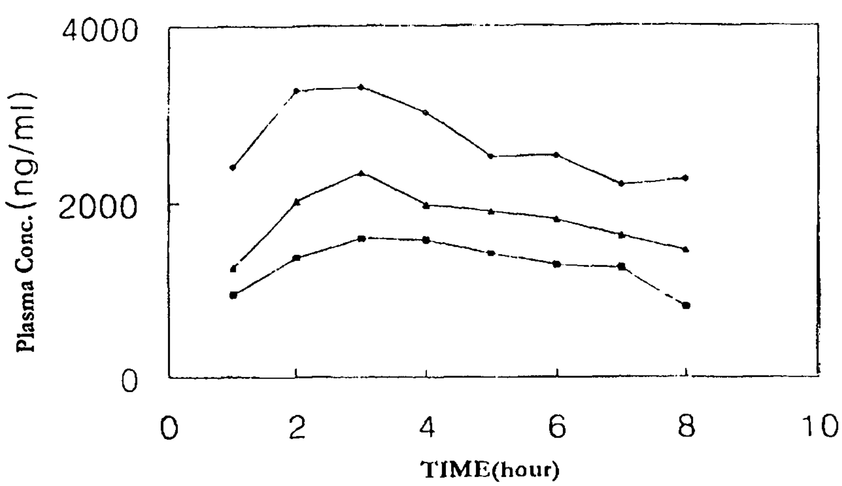

(57) Abstract

Disclosed is a solid dispersed preparation for poorly water–soluble drugs, which is prepared by dissolving or dispersing the poorly water–soluble drugs in an oil, a fatty acid or a mixture thereof, mixing the solution or dispersion in a water–soluble polyol matrix and drying the mixture. The solid dispersed preparation can be formulated into a power formulation or a granule formulation. The solid dispersed preparation is improved in the solubility of poorly water–soluble drugs in the gastro–intestinal tract, resulting in a great increase in the bioavailability of the drugs. In addition, the solid dispersed preparation gives the pharmaceutical solutions to the problems that the conventional semi–solid or liquid preparations possess, enabling medicinally effective, poorly water–soluble compounds to be formulated, molded and processed, quickly and in an economically favorable manner without use of any organic solvent.

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SOLID DISPERSED PREPARATION OF POORLY WATER-SOLUBLE DRUG CONTAINING OIL, FATTY ACID OR MIXTURES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a solid dispersed preparation for poorly water-soluble drugs or biologically active substances. More particularly, this invention relates to a solid dispersed preparation which allows poorly water-soluble drugs to be increased in the uptake efficiency in the gastro-intestinal track and is convenient to make in a pharmaceutical formulation.

Description of the Prior Art

A good many drugs poorly dissolve in water. When being administered to a body, these poorly water-soluble drugs have so low solubility and releasing rate in digestive juices as to retard their absorption, resulting the bioavailability decreased. In order to solve this problem, various preparation methods were developed with the aim of solubilizing these poorly water-soluble drugs and increasing their releasing rates. For instance, there have been reported many methods for improving the bioavailability of drugs, including micronization, formation of micelles by use of surfactant, solvent deposition, utilization of dry elixirs, co-precipitation

by use of inert water-soluble carriers, solid-dispersion and formation of inclusion complexes using cyclodextrins. In conducting these methods, however, the drugs to be administered do not show a constant increase in solubility. Thus, they are problematic in terms of preparation, commercialization, and efficiency.

For the poorly water-soluble drugs, which are also poor in internal uptake, there have been made attempts to enhance their bioavailability upon administration. However, the dosage forms developed thus far, are of semi-solid or liquid form, giving disadvantages in pharmaceutics, especially in formulating, molding and processing.

SUMMARY OF THE INVENTION

We, the inventors made the intensive and thorough research on the formulation of poorly water-soluble drugs, to improve the bioavailability of the drugs upon administration. As a result, we found that the dispersion or solution of the poorly water-soluble drugs in oils, fatty acids or mixtures thereof, followed by mixing with a water-soluble polymer matrix allowed the drugs to efficiently release in the gastro-intestinal tract and the mixture can be formed into a solid form.

Therefore, it is an object of the present invention to provide a solid dispersed preparation which improves the

bioavailability of poorly water-soluble drugs by enhancing the release of the drugs in the gastro-intestinal tract.

It is another object of the present invention to provide a solid dispersed preparation which can be prepared by simple and convenient process with an economical benefit.

According to the present invention, a solid dispersed preparation for poorly water-soluble drugs is prepared by dissolving or dispersing the drugs in an oil, a fatty acid or a mixture thereof, mixing the solution or dispersion in a water-soluble polyol matrix and drying the mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph in which the plasma concentration of cyclosporine is plotted against the times after administrating the solid dispersed preparations of the present invention (closed rectangle and closed triangle) and a commercially available preparation (Neoral, closed lozenge);

Fig. 2 is a graph in which the plasma concentration of aceclofenac is plotted against the times after orally administrating aceclofenac powder (closed circle) and the solid dispersed preparation of the present invention (open circle, oleic acid 5%) to rats;

Fig. 3 is a graph in which the plasma concentration of cyclosporine is plotted against the times after

administrating the solid dispersed preparation of the present invention (closed circle, capsule containing 100 mg of the preparation) and a commercially available preparation (open circle, Airtal capsule 100 mg) to bealgle dogs;

Fig. 4 is a graph in which the plasma concentration of aceclofenac is plotted against the times after orally administrating the solid dispersed preparation of the present invention (closed circle, capsule containing 100 mg of the preparation) and a commercially available preparation (open circle, Airtal capsule 100 mg) to humans; and

Fig. 5 is a graph in which the plasma concentration of cisapride is plotted against the times after orally administrating the solid granular preparations of the present invention (open circle, bead 10 mg) and a commercially available preparation (closed circle, prepulsid 10 mg) to humans.

DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention will be described in detail.

In accordance with the present invention, there is provided a solid dispersed preparation for poorly water-soluble drugs, which is prepared by dispersing or dissolving the drugs in an oil, a fatty acid or a mixture

thereof, incorporating the dispersion or solution into a water-soluble polymer matrix and drying this mixture.

In particular, this invention provides two types of fomulation, i.e., the solid powdery preparation and the solid granular preparation.

The preparation method of the solid dispersed powders comprises the following steps; Dissolving or dispersing the poorly water-soluble drugs in an oil, a fatty acid or the mixture thereof; mixing with the water-soluble polymer matrix; drying the mixture; and grinding the pellet into powders.

In addition, the preparation method of the solid dispersed granules comprises the following steps; Dissolving or dispersing the poorly water-soluble drugs in an oil, a fatty acid or the mixture thereof; mixing with the water-soluble polymer matrix; spraying onto a pharmaceutically acceptable nucleus, resulting the granules. In a preferred embodiment, the pharmaceutically acceptable nucleus may be a sugar sphere.

The solid dispersed powdery preparation or the solid dispersed granular preparation of this invention can be formulated into the pharmaceutically acceptable medicines for internal use such as powders, granules, tablets and capsules.

Hereinafter, the word "solid dispersed preparation" means "solid dispersed powdery preparation", "solid dispersed granular preparation" or the both.

In this regard, the oil, the fatty acid or the mixture thereof may be used alone or in a form of an emulsion or microemulsion inclusive of itself. When dispersing or dissolving poorly water-soluble drugs in the oil, fatty acid or mixture thereof, a surfactant may be added together. Further, the water-soluble polymer matrix may be used alone or in combination with another water-soluble matrix.

Illustrative examples of the oil that can be used in the preparation of the present invention include lipid additives, such as α-bisabolol, stearyl glycerrhetinate, salicylic acid, tocopheryl acetate, a mixture of water, alcohol and Perilla extract, sodium hyaluronate, panthenol, propylene glycol and apple(*Pirus Malus*), propylene glycol and pineapple, ivy (*Hedera halix*) extract and 1,3-B.G, peach (*Prums persica*) leaf extract, hydrolyzed soy flour, wheat (*Triticum Vulgare*) protein, birch (*Betula alba*) extract and 1,3-B.G, burdock (Arctium majus)extract and 1,3-B.G; liposomes; phosphatidylcholines; esters, such as glyceryl stearate, captylic/capric triglyceride, cetyl octanolate, isopropyl myristate, 2-ethylene isopelagonate, di-C12-13 alkyl malate, ceteatyl octanoate, butylene glycol dicaptylate/dicaprate, isononyl isostearate, isostearyl isostearate, coco-captylate/caprate, cetyl octanoate, octyldodecyl myristate, cetyl esters, C10-30 cholesterol/lanosterol ester, hydrogenated castor oil, monoglycerides, diglycerides, and triglycerides; hydrocarbons, such as beeswax, canauba wax, suctose

distearate, PEG-8 beeswax and candelilla (*euphorbia cerifera*) wax; mineral oils such as ceresin and ozokerite; vegetable oils such as macadamia ternifolia nut oil, hydrogenated hi-erucic acid rape seed oil, olive oil, jojoba oil, hybridsunflower (*Helian thus annuus*) oil, neen (*melia azadirachta*) seed oil, dog rose (*rosa canina*) lips oil with preference to mineral oils, squalene, squalane, monoglycerides, diglycerides, triglycerides, medium-chain glyceride, myglyol, cremophor, hydrogenated caster oil, corn oil, Perilla oil, cotton seed oil and lipid-soluble vitamins.

As for the fatty acid, it is preferable to use oleic acid, cetyl alcohol, stearyl alcohol, stearic acid, myristic acid, linoleic acid or lauric acid. More preferable is to use oleic acid, linoleic acid, or isopropyl myristate.

As the water-soluble matrix, polyethylene glycol (PEG), carbowax or polyvinyl pyrrolidone (PVP) is available. Aforementioned water-soluble matrix may be used in combination with other matrixces, examples of which include water-soluble matrices such as gelatin, gum, carbohydrates, celluloses, polyvinyl alcohol, polyacrylic acid, inorganic compounds and mixtures thereof; and enteric matrices such as hydroxypropylmethylcellulose acetyl succinate (HPMCAS), cellulose acetate phthalate, shellac, zein, polyvinyl acetate phthalate, Eudragit L100, Eudragit S100, sodium arginate and poly-L-lysine.

In order to enhance the dispersion or dissolution of poorly water-soluble drugs in the oil, fatty acid or their mixture, a surfactant may be added, which is selected from the group comprising glyceryl stearate, polysorbate 60, polysorbate 80, sorbitan trioleate, sorbitan sesquioleate, sorbitan stearate, PEG-20 glyceryl isostearate, ceteth-25, PEG-60 hydrogenated castor oil, nonoxynol-15, PEG-6-decyltetradeceth-20, dimethicone copolyol, glyceryl diisostearate, ceteth-24, cetearyl alcohol, polyoxylethylene nonyphenyl ether, PEG-40 hydrogenated castor oil, cetyl dimethicone copolyol, polyglyceryl-3-methylglucose distearate, PEG-100 stearate, sorbitan isostearate, sodium lauryl glutamate, disodium cocoamphodiacetate, lauric acid diethanolamide, coconut fatty acid diethanolamide, N,N-bis-(2-hydroxy ethyl)-cocomide, and cocoamidopropyl betain.

The solid dispersed preparation of the present invention can be applied for all the poorly water-soluble drugs and preferably for ketoconazole; itraconazole and its derivatives; cyclosporine; cisapride; acetaminophen; aspirin; acetylsalicylic acid; indomethacin; naproxen; warfarin; papaverine; thiabendazole; miconazole; cinnarizine; doxorubicin; omeprazole; cholecalciferol; melphalan; nifedipine; digoxin; benzoic acid; tryptophan; tyrosine; phenylalanine; aztreonam; ibuprofen; phenoxymethylpenicillin; thalidomide; methyltestosterone; prochlorperazine; hydrocortisone; dideoxypurine

nucleoside; vitamin D_3; sulfonamide; sulfonylurea; p-aminobenzoic acid; melatonin; benzylpenicillin; chlorambucil; diazepin; digitoxin; hydrocortisone butyrate; metronidazole benzoate; tolbutamide; prostaglandin E1(PGE_1); fludrocortisone; griseofulvin; miconazole nitrate; leukotriene B_4 antagonist; propranolol; thephylline; flubiprofen; sodium benzoate; benzoic acid; riboflavin; benzodiazepine; phenobardital; glyburide; sulfadiazine; sulfaethylthiadiazole; sodium diclofenac; aceclofenac; phyniroin; hioridazinehydrochloride; bropirimine; hydrochlorothiazide; fluconazole; acyclovir; bucillamine; ciprofluoxacin; acetyl-L-carnitine; baclofen; sodium alendronate; lovocarnitine; nimodipine or nimodifine; atenolol; provastatin sodium; lovastatin; isotretinoin; etidronate disodium; doxifluridine; fosfomycin calcium; sotepine; epinastine hydrochloride; carvedilol; epinastine hydrochloride; carvedilol; fosinopril; trandolapril; etretinate cap; metergoline; mercaptopurine; vancomycin hydrochloride; cefixime; cefuroxim axetil; dirithramycin; and dadanosin and more preferably for ketoconazole, itraconazole and its derivatives, cisapride, cyclosporine and nifedipine.

Over conventional methods, the present invention has an advantage, in that, the solid dispersed preparation can be prepared with ease and show high efficiency in absorption and release.

First, a poorly water-soluble medicine is homogeneously mixed and dispersed in an oil, fatty acid or their mixture and added in water-soluble polymer matrices molten at room temperature or about 60-80 °C, after which the resulting mixture is cooled rapidly to room temperature and dried in an oven for 12 hours or more. The dried pellet is powdered in a mortar and passed through a sieve to give powder which is uniform in particle size. As aforementioned, when the drug is dispersed or dissolved in the oil, fatty acid or their mixture, the oil, fatty acid or their mixture may be emulsified or micro-emulsified. In this case, a surfactant may be added to the solution.

Alternatively, after the homogeneous dispersion of the poorly water-soluble drug is added in the water-soluble polymer matrix molten at about 60-80 °C, it may be sprayed to pharmaceutically acceptable nucleus to give a granule.

As a consequence of an examination which was made on the solubility of the solid dispersed preparation in distilled water, artificial intestinal juice and artificial gastric juice, the solubility of the solid dispersed preparation is found to be better than those of poorly water-soluble drugs themselves. Particularly, a great advance can be brought into the solubility of poorly water-soluble drugs when they are incorporated into a solid dispersed preparation containing oleic acid or micro-emulsified oleic acid.

The data obtained from the experiments in which the

solid dispersed preparations of the present invention are eluted in artificial gastric juice and artificial intestinal juice, show that the solid dispersed preparations of the present invention are superior to the poorly water-soluble drugs themselves in releasing rate. A significant improvement in releasing rate is observed when a solid dispersed preparation containing oleic acid or microemulsified oleic acid is used. In the artificial intestinal juice, a severer condition in which for drugs to dissolve, rather than in the artificial gastric juice, the improvement in the releasing rate by virtue of the solid dispersed preparation is more apparent.

Through an experiment which is conducted for examining the uptake efficiency of poorly water-soluble drugs in the gastro-intestinal tract, the superiority of the solid dispersed preparation according to the present invention is also demonstrated. Even when only a water-soluble matrix is used, the uptake efficiency of the drugs is minutely increased. In particular, the uptake efficiency of drugs in the gastro-intestinal tract is remarkably improved when they are incorporated in a solid dispersed preparation using oleic acid-containing microemulsions.

In addition, comparison of the plasma concentration of target drug molecule after oral administration between the solid dispersed preparation and conventional preparations, is helpful in understanding the present invention. As a result, similar levels are observed, suggesting that the

solid dispersed preparation of the present invention can substitute for conventional preparations when account is taken of pharmaceutical aspects.

A better understanding of the present invention may be obtained in light of the following examples which are set forth to illustrate, but are not to be construed to limit the present invention.

Following are the compositions of emulsions and microemulsions used in Examples.

EMULSIONS

PREPARATION EXAMPLE I

	Composition (%)
Waxes	
KALCHOL 6870	1.800
EMERSOL 132	1.000
Multi-Wax W-445	1.700
Emulsifiers	
ATLAS G-144	1.800
ATLAS G-610	1.900
ATMOS 370	0.800
KM-105	2.000
Oils	
CRODALAN SWL	1.500

LEXOL GT 865	4.000
NIKKOL CIO	4.000
SEPERIOR JOJOBA OIL	1.000
SF 1202	0.200
KF-96(100CS)	0.300
DRAKEOL 7	5.000
Squalane	2.000
dl-a-Tocopheryl Acetate	0.100
POLYOLPERPOLYMER-2	0.200

Aqueous Phase

DI-WATER	60.852
glycerin	2.000
P.G	7.000
NATURAL EXT.AP	0.500
LUBRAGEL CG	0.200
Carbopo 1940	0.100
KELTROL F	0.020
NaOH	0.028

PREPARATION EXAMPLE II

Waxes

KALCHOL 6870	1.800
EMERSOL 132	1.000
Multi-wax W-445	1.700

Emulsifiers	
RHEODOL AO-15	0.800
RHEODOL MS-162	2.000
RHEODOL TW-S120	1.900
KM-105	2.000
Oils	
CRODALAN SWL	1.500
LEXOL GT 865	5.000
NIKKOL CIO	2.500
Macadamia ternifolia nut oil	1.000
SF 1202	0.300
KF-96(100CS)	0.300
DRAKEOL 7	7.000
Squalane	0.500
dl-a-Tocopheryl Acetate	0.100
POLYOLPERPOLYMER-2	0.100
Aqueous phase	
DI-WATER	61.780
glycerin	2.000
1.3-B.G	6.000
NATURAL EXT.AP	0.300
LUBRAGEL CG	0.200
Carbopol 940	0.100
KELTROL F	0.020
TEA	0.100

PREPARATION EXAMPLE III

Waxes	
KALCHOL 6870	0.500
EMERSOL 132	0.500
Beeswax	0.400
Emulsifiers	
ATLAS G-114	2.200
ATLAS G-610	0.800
ATMOS 370	0.800
KM-105	0.700
Oils	
CRODALAN SWL	0.500
LEXOL GT 865	3.000
NIKKOL CIO	3.000
SUPERIOR JOJOGA OIL	0.500
SR 1202	0.200
KF-96(100CS)	0.100
DRAKEOL 7	3.000
Squalane	0.500
dl-a-Tocopheryl Acetate	0.100
POLYOLPERPOLYMER-2	0.200
Aqueous phase	
DI-WATER	74.146

Glycerin	2.000
P.G	6.000
NATURAL EXT.AP	0.500
LUBRAGEL CG	0.200
Carbopol 940	0.100
KELTROL F	0.020
NaOH	0.0336

PREPARATION EXAMPLE IV

Waxes	
KALCHOL 6870	0.400
EMERSOL 132	0.500
Multi-Wax W-445	0.400
Emulsifiers	
RHEODOL AO-15	0.800
RHEODOL MS-165	2.200
RHEODOL TW-S120	0.800
KM-105	0.600
Oils	
CRODALAN SWL	0.500
LEXOL GT 865	3.000
NIKKOL CIO	2.000
Macadamia ternifolia nut oil	1.000
SF 1202	0.400

DRAKEOL 7	4.500
Squalane	0.500
dl-a-tocopheryl acetate	0.100
POLYOLPERPOLYMER-2	0.100

Aqueous phase

DI-WATER	73.480
glycerin	2.000
1,3-B.G	6.000
NATURAL EXT.AP	0.300
LUBRAGEL CG	0.200
Cabopol	0.100
KELTROL F	0.020
TEA	0.100

MICROEMULSIONS

PREPARATION EXAMPLE V

Waxes

Cetyl Alcohol	3.000

Emulsifiers

NIKKOL HCO-60	5.000
RHEODOL TW-0120	5.000
Cremophor EL	20.000

Oils	
I.P.M	5.000
CAPTEX	5.000
Aqueous phase	
DI-WATER	52.000
Ethanol	5.000

PREPARATION EXAMPLE VI

Emulsifiers	
NIKKOL HCO-60	5.000
RHEODOL TW-0120	5.000
Cremophor EL	5.000
Oils	
I.P.M	5.000
Lanolin oil	5.000
CAPTEX	5.000
Aqueous phase	
DI-WATER	50.000

PREPARATION EXAMPLE VII

Surfactant	
LABRASOL	15.000

Surfactant Aid	
Polyglyceryl oleate	5.000
PLURL OLEIQUE	5.000
Oil phase	
LABRAFIL M1994CS	4.500
Sub-Solvent	
Transcutol	5.000
Aqueous phase	
Phosphate buffer(pH 6)	64.500

PREPARATION EXAMPLE VIII

Oil phase	
GELUCIRE 44/14	11.429
GELUCIRE 48/09	11.429
Surfactant	
LABRAFAC CM 10	10.714
Surfactant Aid	
LAUROGLYCOL	7.143
Transcutol	59.285

PREPARATION EXAMPLE IX

Aqueous Phase

Water (Buffer)	57,050
Physiological Saline Solution	4,000
Glucose	1,000
Propylene Glycol PEG 300,400	5,000
Glycerol	5,000

Oil Phase

Fatty Acid Esters	5,000
Modified Vegetable Oil	0.500
Silicon Oil	0.500

Surfactant Aid

Long Chain Alcohol	3,750
Glycol Derivative	2,500
Propylene Glycol Derivative	1,200
Polyglycerol Derivative	4,500

Surfactant

Non-ionic Surfactant	10,000

PREPARATION EXAMPLE X

Oil Phase

Oleic Acid	6,250

Surfactant	
Tween 80	12,500
Surfactant Aid	
Transcutol	8,750
Aqueous Phase	
Water	72,500

PREPARATION EXAMPLE XI

Oil Phase	
Captex	5,000
Surfactant	
Cremophor	12,500
Surfactant Aid	
Transcutol	6,250
Aqueous Phase	
Water	76,250

COMPARATIVE EXAMPLE I

After being melted at about 70 °C, 90 g of PEG 6000 was added with 10 g of ketoconazole, cooled rapidly to room

temperature and dried in an oven for 12 hours or more. The dried solid dispersed preparation was milled in a mortar and passed through a sieve to give a powder which was uniform in particle size.

EXAMPLE I

In 5 g of oleic acid were homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added into 85 g of PEG 6000 which was molten at about 70 °C. After being cooled rapidly to room temperature and dried in an oven for 12 hours or more, the dried solid dispersed preparation was milled in a mortar and passed through a sieve to give a powder which was uniform in particular size.

EXAMPLE II

In 5 g of oleic acid and 5 g of Tween 80 were homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE III

In 5 g of isopropyl myristate was homogeneously mixed

and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE IV

In 5 g of liquid paraffin was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE V

In 5 g of cremophor was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE VI

In 5 g of cremophor and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was

obtained in the same procedure as in Example I.

EXAMPLE VII

In 5 g of isopropyl myristate and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE VIII

In 5 g of liquid paraffin and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE IX

In a microemulsion containing 5 g of cremophor, 5 g of oleic acid, 35 g of alcohol and 1 g of transcutol was homogeneously dissolved and dispersed 10 g of ketoconazole, followed by evaporating the alcohol. The solid residue was, then, added in 43 g of PEG 6000 molten at about 70 °C.

Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE X

In a microemulsion containing 5 g of cremophor, 5 g of oleic acid and 1 g of transcutol was dissolved 10 g of ketoconazole which was, then, dispersed in 35 g of distilled water, followed by evaporating the distilled water in an oven. The solid residue was added in 43 g of PEG 6000 molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XI

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of ketoconazole. 40 g of hydroxypropylmethylcellulose, an enteric matrix, was added in 40 g of PEG 6000 molten at 70 °C. Using the mixture of the above two solutions, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of itraconazole which was, then, added in 80

g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XIII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of itraconazole. 40 g of hydroxypropylmethylcellulose, an enteric matrix, was added in 40 g of PEG 6000 which was molten at 70 °C. Using the mixture of the above two solutions, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XIV

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of an itraconazole derivative (Dong-A Pharmacy Co., Ltd., Korea) which was, then, added in 80 g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XV

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of cyclosporine which was, then, added in 80

g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XVI

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of cyclosporine. 40 g of hydroxypropylmethylcellulose, an enteric matrix, was added in 40 g of PEG 6000 which was molten at 70 °C. Using the mixture of the above two solutions, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XVII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of cisapride which was, then, added in 80 g of PEG 6000 which was molten at 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XVIII

In 5 g of oleic acid and 5 g of Tween 80 was homogeneously mixed and dispersed 10 g of cisapride which was, then, added in 80 g of PEG 6000 which was molten at 70

°C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XIX

In a microemulsion containing 10 g of cremophor, 5 g of oleic acid and 7 g of transcutol was homogeneously dissolved and dispersed 10 g of itraconazole, followed by evaporating the alcohol in an oven. The solid residue was, then, added in 43 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XX

In a microemulsion containing 10 g of cremophor, 4 g of captex and 5 g of transcutol was homogeneously dissolved and dispersed 10 g of cyclosporine, followed by evaporating the alcohol in an oven. The solid residue was, then, added in 43 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XXI

In a microemulsion containing 10 g of cremophor, 5 g of oleic acid and 7 g of transcutol was homogeneously

dissolved and dispersed 10 g of cisapride, followed by evaporating the alcohol in an oven. The solid residue was, then, added in 43 g of PEG 6000 which was molten at about 70 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XXII

In 5 g of oleic acid was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of molten PVP. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

EXAMPLE XXIII

In a microemulsion containing 5 g of oleic acid was homogeneously mixed and dispersed 10 g of ketoconazole which was, then, added in 80 g of molten PVP. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example I.

COMPARATIVE EXAMPLE II

After being melted at about 70 °C, 2.5 g of molten PEG 6000 was added with 1.75 g of aceclofenac, cooled rapidly to room temperature and dried in a freeze-drier for 24 hours or

more. The dried solid dispersed preparation was finely milled in a grinder and passed through a sieve to give a powder which was uniform in particle size.

EXAMPLE XXIV

In 0.25 g of oleic acid and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac, and then, the solution was added in 2.5 g of PEG 6000 which was molten at about 75 °C. After being cooled rapidly to room temperature
And dried in a freeze-drier for 24 hours or more, the dried solid dispersed preparation was finely milled in a grinder and passed through a sieve to give a powder which was uniform in particle size.

EXAMPLE XXV

In 0.25 g of cemophor and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac which was, then, added in 2.5 g of PEG 6000 which was molten at about 75 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example XXIV.

EXAMPLE XXVI

In 0.25 g of labrasol and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac which was, then, added in 2.5 g of PEG 6000 molten at about 75 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example XXIV.

EXAMPLE XXVII

In 0.25 g of transcutol and 0.50 g of Tween 80 was homogeneously mixed and dispersed 1.75 g of aceclofenac which was, then, added in 2.5 g of PEG 6000 molten at about 75 °C. Using this mixture, a dispersed powdery preparation was obtained in the same procedure as in Example XXIV.

EXAMPLE XXVIII

A mixture of 10 g of aceclofenac, 2.5 g of oleic acid, 2.5 g of Tween 80, 5 g of talc and 10 g of PEG 6000 was heated at about 80 °C and homogeneously dispersed in 150 ml of an alcohol. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting solution was sprayed at a rate of 4 ml/min onto 35 g of sugar spheres to give a solid dispersed granule.

EXAMPLE XXIX

10 g of aceclofenac, 2.5 g of oleic acid, 2.5 g of Tween

80, 3 g of talc, 25 g of Eudragit (Rhompharm, Germany) RS30D and 25 g of Eudragit L30D were homogeneously mixed. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting solution was sprayed at a rate of 4 ml/min onto 35 g of the sugar spheres prepared in Example XXVIII.

EXAMPLE XXX

10 g of aceclofenac, 2.5 g of oleic acid, 2.5 g of Tween 80, 3 g of talc and 50 g of Eudragit RS30D were homogeneously mixed. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting mixture was sprayed at a rate of 4 ml/min onto the sugar spheres prepared in Example XXVIII.

EXAMPLE XXXI

A mixture of 2.5 g of cisapride, 2.5 g of oleic acid, 2.5 g of Tween 80, 5 g of talc and 23 g of PEG 6000 was heated at about 80 °C and added with 150ml of a mixture of acetone and water(acetone:water, 1:1). With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting mixture was sprayed at a rate of 4 ml/min onto 100 g of sugar spheres.

EXAMPLE XXXII

2.5 g of cisapride, 2.5 g of oleic acid, 2.5 g of Tween 80, 3 g of talc, 25 g of Eudragit RS30D and 25 g of Eudragit

L30D were homogeneously mixed in 150ml of acetone. With the aid of a fluid bed-coating machine (nozzle; 0.8 mm), the resulting mixture was sprayed at a rate of 4 ml/min onto 70 g of the sugar spheres prepared in Example XXXI.

EXAMPLE XXXIII

Aceclofenac, lactose, starch and talc were mixed to give a tablet in accordance with the established method. 2.5g of aceclofenac, 2.5g of oleic acid, 2.5g of tween 80, 3g of talc, 25g of Eudragit RS30D and 25g of Eudragit L30D were homogeniously mixed. With the aid of a fluid bed-coating machine (nozzle;0.8mm), the resulting mixture was sprayed at a rate of 4ml/min onto the said tablets to obtain a solid dispersed tablet.

EXAMPLE XXXIV

Cisapride, lactose, starch and talc were mixed to give a tablet in accordance with the established method. 2.5g of cisapride, 2.5g of oleic acid, 2.5g of tween 80, 25g of Eudragit RS30D and 25g of Eudragit L30D were homogeniously mixed. With the aid of a fluid bed-coating machine (nozzle;0.8mm), the resulting mixture was sprayed at a rate of 4ml/min onto the said tablets to obtain a solid dispersed tablet.

EXPERIMENT I : The Drug Solubility of Solid Dispersed Preparation In Water and Artificial Intestinal Juice

In this experiment, the solubility of poorly water-soluble drugs in water and artificial intestinal juice was investigated for the solid preparations obtained in Comparative Example and Examples. In this regard, suspensions of 2 g of the solid dispersion preparations of this invention in water or artificial intestinal juice were filtrated through a 0.2 μm filter paper (Millipore, Waters, Milford, MA, USA) and the filtrate was diluted for the convenient quantification of the drugs. The solubility results are given in Table 1.

TABLE 1

Solubility of Ketoconazole in distilled water and artificial intestinal juice

Solid Dispersed Preparatio	Solubility (μg/mP)	
	DI-Water	Artificial Intestinal Juice
Ketoconazole Powder	0.10	2.08
Comparative Example	3.77	-
Example I	41.4	44.8
Example II	73.9	-
Example III	2.47	-
Example IV	2.28	-
Example V	8.02	-

Example VI	12.0	-
Example VII	6.31	-
Example VIII	12.2	-
Example IX	72.8	50.7
Example X	63.6	37.8

As apparent from the data of Table 1, the solubility of the drugs in distilled water was significantly improved when they were incorporated in solid dispersed preparations containing oleic acid. Particularly, the drugs in the solid dispersed preparations prepared from microemulsions containing oleic acid showed a great advance in the solubility in water as well as in artificial intestinal juice.

EXPERIMENT II: The drug-releasing Rate of Solid Dispersed Preparations in Artificial Gastric and Intestinal Juices

The solid dispersed preparations comprising ketoconazole or cisapride, respectively, obtained in Examples, were tested for releasing rates in artificial gastric juice and artificial intestinal juice.

According to the paddle process described in Korean Pharmacopoeia VI (KP VI), this releasing test was carried out in artificial gastric juice and artificial intestinal juice at 37±0.5 °C while the paddle was rotated at 50 rpm.

At an interval of a predetermined period of time, samples were taken from the artificial juices and filtered through 0.2 μm Millipore paper and the filtrates were measured for plasma concentration of drug. The releasing levels and percentages of the poorly water-soluble drugs against artificial gastric and intestinal juices are given in Tables 2 and 3.

TABLE 2

Releasing Level (μg/ml) and Percentage (%) of Poorly water-soluble Drugs in Artificial Gastric Juice

Prep.	Time (hours)								
	0.25	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0
Keto. Powder	432 (72.0)	437 (72.8)	436 (72.7)	436 (72.6)	434 (72.4)	439 (73.2)	437 (72.7)	435 (72.5)	437 (72.7)
Exmp. I	46.7 (95.9)	49.4 (101.5)	50.5 (103.8)	50.8 (104.3)	50.8 (104.3)	50.6 (104.1)	50.4 (103.6)	50.5 (103.7)	50.5 (103.8)
Exmp. II	49.5 (108.9)	51.6 (113.5)	52.7 (115.9)	53.1 (116.9)	53.5 (117.7)	53.4 (117.4)	52.9 (116.4)	82.9 (116.4)	53.0 (116.6)
Exmp. III	51.6 (107.5)	51.6 (107.6)	52.7 (109.9)	53.1 (110.8)	53.5 (111.6)	53.8 (112.2)	53.0 (110.6)	53.4 (111.3)	53.0 (110.7)
Exmp. IV	51.7 (112.3)	51.4 (111.8)	51.4 (111.7)	51.2 (111.3)	51.6 (112.2)	52.0 (113.0)	51.5 (111.9)	50.9 (110.6)	51.8 (112.6)
Exmp. V	50.3 (111.5)	50.9 (112.7)	50.4 (111.7)	50.7 (112.3)	50.9 (112.7)	50.8 (112.4)	50.8 (112.4)	60.0 (112.9)	50.7 (112.3)
Exmp. VI	45.8 (99.0)	46.3 (100.0)	46.2 (99.8)	46.2 (99.9)	46.2 (99.9)	45.8 (98.9)	45.6 (98.5)	45.1 (97.5)	45.8 (99.1)
Exmp. VII	48.8 (100.4)	48.8 (100.4)	48.9 (100.4)	48.9 (100.6)	49.0 (100.9)	49.9 (102.5)	49.9 (102.7)	50.2 (103.2)	50.1 (102.9)
Exmp. VIII	46.5 (104.3)	45.8 (102.8)	45.9 (102.9)	45.5 (102.1)	46.4 (104.2)	46.4 (104.1)	45.8 (102.8)	45.3 (101.7)	45.6 (102.3)
Cisa-pride Powder	-	5.249 (51.57)	-	5.492 (54.51)	5.914 (58.63)	6.243 (61.81)	6.173 (61.22)	-	6.446 (65.80)

Exmp. XVII	-	8.83 (85.27)	-	8.74 (84.09)	9.12 (87.9)	8.79 (84.54)	9.13 (87.81)	-	9.30 (89.47)
Exmp. XVIII	-	9.84 (103.2)	-	9.74 (102.1)	10.03 (105.2)	9.93 (104.3)	9.76 (102.4)	-	9.68 (101.5)

As shown in Table 2, ketoconazole, although it can be released in the artificial gastric juice to an extent because of its acidic property, is relatively further improved in the releasing level and percentage when it is incorporated in the oleic acid-containing solid dispersed preparations. Therefore, these data are consistent with those of Experiment I. In the meanwhile, cisapride was released to an extent by virtue of its solubility, but also considerably increased in the releasing properties when it was used in the solid dispersed preparations of the present invention.

TABLE 3

Releasing Level (μg/ml) and Percentage (%) of Poorly water-soluble Drugs in Artificial Intestinal Juice

Prep.	Time (hours)								
	0.25	0.5	0.75	1.0	1.5	2.0	3.0	4.0	6.0
Ketoco azole Powder	1.84 (0.092)	1.89 (0.095)	1.91 (0.096)	1.92 (0.096)	1.94 (0.97)	1.99 (0.099)	1.98 (0.099)	2.05 (0.103)	2.08 0.104
C.Exmp	3.05 (4.99)	3.38 (5.53)	3.69 (6.05)	3.71 (6.08)	3.75 (6.14)	3.84 (6.29)	3.87 (6.33)	3.87 (6.34)	4.32 (7.08)
Exmp. I	4.89 (10.05)	5.14 (10.56)	5.68 (11.67)	5.80 (11.91)	5.17 (10.61)	5.2 (10.68)	5.2 (10.68)	5.32 (0.92)	6.00 (12.33
Exmp.	3.55	3.61	3.71	3.98	3.7	3.97	4.09	4.11	4.29

II	(7.82)	(7.94)	(8.15)	(8.75)	(8.13)	(8.73)	(8.98)	(9.04)	(9.42)
Exmp. III	1.44 (3.00)	1.45 (3.04)	1.46 (3.05)	1.67 (3.47)	1.77 (3.69)	1.92 (3.99)	1.95 (4.06)	2.14 (4.47)	2.36 (4.92)
Exmp. IV	1.03 (2.23)	1.27 (2.76)	1.31 (2.84)	1.36 (2.95)	1.45 (3.15)	1.48 (3.21)	1.57 (3.41)	1.63 (3.54)	1.69 (3.67)
Exmp. V	2.21 (4.89)	2.23 (4.94)	2.21 (5.03)	2.27 (5.10)	2.31 (5.15)	2.33 (5.46)	2.47 (5.26)	2.38 (5.34)	2.40 (5.30)
Exmp. VI	2.78 (6.00)	2.53 (5.47)	2.42 (5.23)	2.54 (5.49)	2.19 (4.72)	2.41 (5.21)	2.3 (4.97)	2.34 (5.06)	2.45 (5.29)
Exmp. VII	2.09 (4.28)	2.03 (4.16)	2.1 (4.31)	2.20 (4.51)	2.07 (4.26)	2.2 (4.52)	2.16 (4.43)	2.08 (4.26)	2.08 (4.26)
Exmp. VIII	2.26 (5.07)	2.51 (5.61)	2.42 (5.42)	2.64 (5.92)	2.58 (5.77)	2.57 (5.76)	2.42 (5.41)	2.52 (5.64)	2.59 (5.81)
Exmp. IX	3.55 (10.70)	3.95 (11.89)	4.12 (12.41)	4.27 (12.86)	4.28 (12.88)	4.34 (13.08)	4.38 (13.19)	4.38 (13.20)	4.36 (13.13
Exmp. X	2.37 (6.75)	2.48 (7.05)	2.39 (6.79)	2.14 (6.08)	2.78 (7.92)	2.67 (7.60)	3.42 (9.72)	3.61 (10.27)	3.63 (10.33
Cisa-pride Powder	-	0 (0)	-	0 (0)	0 (0)	0 (0)	0.005 (0.047)	0.028 (0.27)	0.0745 (0.618
Exmp. XVII	-	2.43 (20.15)	-	3.22 (26.7)	2.70 (22.42)	2.66 (22.1)	2.64 (21.94)	3.10 (25.73)	3.99 (33.12
Exmp. XVIII	-	6.34 (63.0)	-	6.75 (67.01)	6.56 (65.15)	6.55 (65.05)	6.69 (66.46)	6.74 (66.9)	6.96 (69.05

The effect of the solid dispersed preparations on improving the releasing rates of the two drugs is more apparent in the artificial intestinal juice, a more difficult condition in which for the two drugs to dissolve. As shown in Table 3, the releasing properties of drugs are better when they are incorporated in the solid dispersed preparations using fatty acid and oil than when they are used alone. A better improvement effect was obtained from the solid dispersed preparations containing oleic acid. Further, the use of microemulsified oleic acid brought

about a great advance in the releasing properties.

In a ddition, the solid dispersed preparations containing itraconazole, its derivatives, and cyclosporine, respectively, was tested for the releasing properties in the artificial gastric and intestinal juices. The results are given in Table 4. Also, the data of Table 4 demonstrate that the drugs in the solid dispersed preparations are superior to the drugs alone in the releasing properties.

TABLE 4

Releasing Level (μg/ml) of Poorly water-soluble Drugs in Artificial Gastric and Intestinal Juice

Prep.	Time (Min)											
	Artificial Gastric Juice						Artificial Intestinal Juice					
	5	15	30	60	90	120	5	15	30	60	90	120
Itra[1]	14.4	16.4	17.4	16.7			0.05	0.05	0.05	0.05	-	-
Exmp.XII	292	293	321	331			130	95.0	146	102	-	-
Exmp.XIII	138	160	179	192			246	214	204	203	-	-
Itra Drv.[2]	96.9	156.2	189.2	211.0	216.7		-	-	-	-	-	-
Exmp.XIV	204.5	198.6	232.6	252.9	259.8		-	-	-	-	-	-
Cyclo.[3]	-	-	1.8	1.7	-	1.9	-	-	2.1	2.3	-	2.5
Exmp.XV	-	-	111.4	94.8	-	71.7	-	-	102.8	99.4	-	91.0
Exmp.XVI	-	-	11.1	10.8	-	9.8			595.0	66.3	-	56.7

[1] Itrakonazole powder

[2] Itrakonazole derivative

[3] Cyclosporine

EXPERIMENT III: Uptake of Poorly water-soluble Drugs in

Rabbit 's Gastrointestinal tract

The solid dispersed preparations containing ketoconazole, prepared from Examples, were tested for the uptake in rabbit's gastrointestinal tract. The results are given in Table 5.

In this regard, first, a rabbit was killed by introducing air in its ear vein and its stomach, duodenum, jejunum, ileum, colon and rectum were excised and washed with physiological saline solution at 37 °C. These organs were fixed between the receptor and donor of a Franz diffusion cell. In the receptor, a physiological saline solution warmed to 37 °C was poured, and stirred with a magnetic stirrer while the solid dispersed preparations obtained in Examples were added in the donor. Samples were harvested from the receptor at predetermined times for 6 hours while the receptor was supplemented with a fresh physiological saline solution in order to constantly maintain the total volume in the receptor. The samples taken were measured for their plasma concentrations of drugs.

TABLE 5

Uptake ($\mu g/cm^2$) of Ketoconazole in Rabbit's GI tract

Prep.	Time (hours)							
	0.3	0.67	1	1.5	2	3	4	6
C. Exmp	0	0.92	2.45	4.50	4.67	5.15	5.56	5.95

Exmp. I	0	1.72	3.44	5.46	9.10	10.2	11.6	13.2
Exmp. II	0.50	2.78	5.50	9.83	15.5	16.3	18.6	22.4

It is apparent from the data of Table 5 that the uptake of the drug in the GI tract is much better when it is incorporated in the solid dispersed preparation using oleic acid than when it is incorporated in the conventional dispersed preparation which uses a water-soluble matrix merely. Particularly, a significant improvement in the uptake of ketoconazole in the GI tract was brought about by the use of the solid dispersed preparations obtained from microemulsions containing oleic acid. These results are consistent with those of Experiment I and II.

EXPERIMENT IV: Comparison of the Plasma Concentration of Drugs Formulated into a Solid Dispersed Preparation and Commercially Available Ones

Before an experiment, male mice (Sprague-Dawley lineage) weighing 250-310 g, purchased from the Korea National Institute of Health, were adapted to new circumstances for 1-2 weeks. After the mice, which were starved from one day before the experiment, were etherized, their left femoral arteries were inserted with cannulas connected to syringes containing 80 IU/ml of heparin. After 2 hours, the mice came out of the ether and were administered with a suspension of the cyclosporine-

containing solid dispersed preparation of the present invention and a commercially available preparation with the aid of a sonde. At an interval of a predetermined period of time, blood was taken from the left femoral arteries and measured for the plasma concentration of drug.

With reference to Fig. 1, the cyclosporine level in blood are plotted against the times after administration for the solid dispersed preparations of the present invention and a commercially available preparation. As shown in the graph, the plasma concentration of the solid dispersed preparations according to the present invention are similar to that of the commercially available preparation, Neoral. Although being a little bit lower concentration than that of Neoral as a whole, the solid dispersed preparations according to the present invention are thought to have the initiatives to substitute for the conventional preparations which contain liquid drugs, in a pharmaceutical aspect.

Solid dispersed preparations according to the present invention and a commercial available preparation, all containing itraconazole as a medicinally effective ingredient, were administered to beagle dogs in an oral route. After a blood sample was taken from their veins at an interval of a predetermined period of time, the plasma concentration of drug was measured. The results are given in Table 6.

TABLE 6

Itraconazole Level (μg/ml) in Blood According to Times

Prep.		Time (hours)							
		1	2	3	4	6	8	10	24
Starved Dog	Exmp. XII	0	0.03	0.03	0.04	0.03	0.02	0.02	0.02
	Exmp. XIII	0.02	0.06	0.07	0.08	0.10	0.07	0.04	0.03
	Purchased	0	0.06	0.04	0.03	0.09	0.03	0.06	0.03
Non-starved	Exmp. XIII	0.12	0.41	0.38	0.44	0.43	0.43	0.42	0.36
	Purchased	0.30	0.60	0.79	0.58	0.54	0.44	0.41	0.30

As apparent from Table 6, a similar pharmacokinetic pattern was observed between the plasma concentration of itraconazole from the solid dispersed preparations and from the conventional preparation (itazol) when starved beagle dogs were administered therewith, while the lower value shown in case the preparation of Examp. XII was administrated. In non-starved beagle dogs, the drug reached a high maximal level in blood within a fast period of time when the commercially available preparation was administered whereas the preparation of Example XIII maintained the plasma concentration of drug constantly, owing to its solubilization in the gastro-intestinal tract.

EXPERIMENT V: Solubility of Aceclofenac in Various Vehicles

Excess aceclofenac was added in 5 ml of a vehicle in a test tube, which was then voltexed to an extent that the drug

was not dissolved further, and incubated for 3 days in a 37 °C water bath. The resulting solution was filtrated through a 0.2 μm filter paper (Millipore, Waters, Milford, MA, USA) and the filtrate was diluted for the convenient quantification of the drug. The solubility results are given in Table 7.

Table 7

Solubility of Aceclofenac in Vehicles

Vehicles	Solubility (mg/mP)
Transcutol	149.34
Labrasol	114.83
Tween 80	98.70
Tween 20	85.71
Cremophor EL	40.92
Cremophor RH40	23.34
Oleic acid	4.59
Linoleic acid	5.44
Triacetin	18.01
Castor oil	13.21
Sesame oil	2.83
Corn oil	2.20
Mineral oil	0.34

As apparent from the data of Table 7, large values are found in the solubility of aceclofenac in fatty acids, triacetin, castor oil and cremophor. Particularly, the drug is dissolved at great amounts in transcutol, labrasol and Tweens.

EXPERIMENT VI: Releasing of Aceclofenac in Solid Dispersed Preparations Against Artificial Gastric and Intestinal

Juices

The solid dispersed preparations comprising aceclofenac, obtained in Examples XXIV to XXVII, were tested for releasing properties against artificial gastric juice and artificial intestinal juice in a similar manner to that of Experimental Example II.

The releasing levels and percentages of the poorly water-soluble drugs against artificial gastric and intestinal juices are given in Tables 8 and 9.

Table 8

Releasing Level (μg/ml) and Percentage (%) of Aceclofenac in Artificial Gastric Juice

Prep.	Time (hours)						
	0.25	0.5	0.75	1.0	1.5	2.0	3.0
Aceclofenac Powder	0.46 (0.23)	0.53 (0.26)	0.57 (0.28)	0.60 (0.30)	0.61 (0.31)	0.69 (0.34)	0.73 (0.37)
Exmp. XXIV	1.01 (0.51)	1.16 (0.58)	1.29 (0.65)	1.33 (0.67)	1.35 (0.67)	1.43 (0.72)	1.38 (0.69)
Exmp. XXV	1.68 (0.84)	2.38 (1.19)	2.43 (1.22)	2.51 (1.25)	2.68 (1.34)	2.65 (1.33)	2.70 (1.35)
Exmp. XXVI	1.61 (0.80)	1.88 (0.94)	1.96 (0.98)	1.98 (0.99)	1.99 (1.10)	1.95 (0.98)	2.08 (1.04)
Exmp. XXVII	1.76 (0.88)	2.04 (1.02)	2.36 (1.18)	2.51 (1.26)	2.61 (1.30)	2.70 (1.35)	2.63 (1.31)
Airtal	0.93 (0.46)	1.02 (0.51)	1.18 (0.59)	1.23 (0.61)	1.32 (0.66)	1.34 (0.67)	1.39 (0.70)

As shown in Table 8, the releasing of aceclofenac in the artificial gastric juice was much improved when it was

in the solid dispersed preparations of the present invention relatively to the other preparations.

TABLE 9

Releasing Level (μg/ml) and Percentage (%) of Aceclofenac in Artificial Intestinal Juice

Prep.	Time (hours)							
	0.25	0.5	0.75	1.0	1.5	2.0	3.0	5.0
Aceclo. Powder	88.37 (44.19)	117.34 (58.67)	121.65 60.82	126.64 (63.32)	128.10 (64.05)	131.70 (65.85)	136.55 (68.27)	136.55 (68.28)
Exmp. XXIV	152.97 (76.49)	157.43 (78.72)	161.90 80.95	160.40 (80.20)	162.66 (81.33)	164.09 (82.05)	165.27 (82.63)	166.71 (83.35)
Exmp. XXV	151.72 (75.86)	163.33 (81.67)	161.72 80.86	163.11 (81.55)	162.26 (81.13)	165.57 (82.79)	166.16 (83.08)	166.16 (83.08)
Exmp. XXVI	148.21 (74.10)	152.40 (76.20)	154.58 77.29	154.95 (77.47)	154.49 (77.24)	155.48 (77.74)	157.97 (78.99)	159.74 (79.87)
Exmp. XXVII	138.83 (69.41)	150.41 (75.21)	155.85 77.92	161.51 (80.75)	161.63 (80.81)	163.29 (81.64)	164.22 (82.11)	167.36 (83.68)
Airtal	133.76 (66.88)	136.54 (68.27)	136.62 68.31	137.70 (68.85)	142.55 (71.28)	145.72 (72.86)	143.66 (71.83)	142.34 (71.17)

As known from Table 9, aceclofenac, although it can be released in the artificial gastric juice to an extent because of its basic property, is relatively further improved in the releasing level and percentage when it is formulated into the solid dispersed preparation.

EXPERIMENT VII: Comparison of Plasma Concentration of Aceclofenac Between Solid Dispersed Preparation and Conventional Ones

Before an experiment, male mice (Sprague-Dawley lineage) weighing 250-310 g, purchased from the Korea National Institute of Health, were adapted to new circumstances for 1-2 weeks. After the mice, which were starved from one day before the experiment, were etherized, their left femoral arteries were inserted with cannulas connected to syringes containing 50 IU/ml of heparin. After 2 hours, the mice came out of the ether and were administered with a suspension of the aceclofenac-containing solid dispersed preparation of the present invention and a aceclofenac powder with the aid of a sonde. At an interval of a predetermined period of time, blood was taken from the left femoral arteries and measured for the plasma concentration of the drug.

In the meanwhile, the aceclofenac-carrying solid dispersed preparations of the present invention and a commercial available preparation were orally administered to beagle dogs and volunteers. At predetermined times after oral administration, blood was taken from the beagle dogs and the volunteers and measured for the drug levels.

After the oral administration of the aceclofenac-carrying solid dispersed preparations of the present invention, an aceclofenac powder and a commercial available preparation to mice, beagles dogs and volunteers, the plasma concentration of the drug with time were compared and are plotted in Figs. 2 to 4.

As shown in the graphs, the solid dispersed preparations of

the present invention maintain higher levels of aceclofenac in blood for all of the testees than the commercially available preparation. In addition, the use of the solid dispersed preparation according to the present invention was affirmed to increase the maximal value of plasma concentration and area under the curve, which are pharmacokinetic parameters, by 1.5-6 times.

After the oral administration of the aceclofenac-carrying solid dispersed preparation of the present invention, a commercially available preparation and an aceclofenac powder, the plasma concentration of aceclofenac was monitored with time and the results are given in Tables 10 to 12, below.

TABLE 10

Aceclofenac Level (μg/ml) in Blood of Rat

Prep.	Time (hours)								
	0.25	0.5	0.75	1	1.5	2	3	4	6
Exmp. XXIV	11.11	14.30	12.96	8.01	4.45	3.38	2.60	0.70	0.85
Aceclo. Powder	1.85	0.71	0.44	0.15	0.03	0.16	0.21	0.27	0.13

It is apparent from the data of Table 10 that the aceclofenac level in blood is significantly improved when the drug is administered by use of the preparation of the present invention relative to when aceclofenac is administered alone.

TABLE 11

Plasma Concentration (μg/ml) of Aceclofenac

in Beagle Dogs

Prep.	Time (hours)											
	0.25	0.5	0.75	1	1.5	2	3	5	7	9	12	24
Exmp. XXIV	4.9	41.1	74.1	81.8	93.0	96.5	71.1	49.4	32.2	21.6	11.1	1.3
Airtal	4.6	15.1	28.9	35.7	50.8	43.2	31.9	16.6	8.8	6.4	3.9	0.8

The data of Table 11 demonstrate that the aceclofenac-carrying solid dispersed preparation of the present invention is superior to the conventional preparation in the plasma concentration.

TABLE 12

Plasma Concentration (μg/ml) of Aceclofenac

in Human Blood

Prep.	Time (hours)										
	0.25	0.5	0.75	1	1.5	2	3	5	7	9	12
Exmp. XXIV	0.16	4.67	10.98	18.12	12.99	6.93	2.97	1.02	0.67	0.56	0.42
Airtal	0.85	1.75	3.84	5.51	5.48	8.34	3.44	0.48	0.29	0.14	0.10

As apparent from the data of Table 12, higher levels of aceclofenac in blood are maintained when the solid dispersed preparation of the present invention is administered than when the commercially available preparation is used.

EXPERIMENT VIII: Releasing of Cisapride From Solid

Dispersed Preparations Against Artificial Gastric and Intestinal Juices

The solid dispersed preparation comprising cisapride, obtained in Examples XXXI, was tested for releasing properties against artificial gastric juice and artificial intestinal juice in a similar manner to that of Experimental Example II.

The releasing levels and percentages of the poorly water-soluble drugs against artificial gastric and intestinal juices are given in Table 13.

TABLE 13

Releasing Level (μg/ml) and Percentage (%) of Cisapride in Artificial Gastric and Intestinal Juice

Juice	Time (hour)									
	0.5	1.0	1.5	2.0	3.0	4	5	6	8	12
Gastric	14.9	26.2	32.0	43.4	58.5	–	84.7	–	–	–
Intestina	8.1	16.5	25.2	38.1	48.5	62.1	–	72.7	87.8	98.0

The amount of the drug released from the solid dispersed preparation was increased almost linearly in both artificial gastric and intestinal juices, showing a zero order-like kinetics.

EXPERIMENT IX: Comparison of Plasma Concentration of

Cisapride Between Solid Dispersed Preparation and Commercially Available Ones

At an interval of a predetermined period of time after the oral administration of beagle dogs with the cisapride-carrying solid dispersed preparation obtained in Example XXXII and a commercially available preparation, blood was taken from the testees and measured for plasma concentration of drug.

With reference to Fig. 5, the cisapride levels in blood are plotted against the times after administration for the solid dispersed preparations of the present invention and a commercially available preparation, Prepulsid tablet. As shown in the graph, the plasma concentration of the solid dispersed preparations according to the present invention are greatly improved relative to that of the commercially available preparation. These drug concentrations are numerically shown in Table 14, below.

TABLE 14

Plasma Concentration of Cisapride (μg/ml)

in Beagle Dog

Prep.	Time (hours)											
	0.5	0.75	1	1.25	1.5	2	3	5	7	9	12	24
Exmp. XXXII	48	80	68	82	97	151	271	284	152	104	83	63
Prepulsid	49	61	70	83	102	184	201	134	75	58	46	41

As shown in Table 14, the plasma concentration of cisapride is maintained at higher levels when the solid dispersed preparation of the present invention is used than when the conventional preparation.

As described hereinbefore, the solid dispersed preparations of the present invention are improved in the solubility of poorly water-soluble drugs in the gastro-intestinal tract, in detail, the releasing of the drugs against the gastric and intestinal juices, resulting in a great increase in the bioavailability of the drugs. In addition, the solid dispersed preparations of the present invention give the pharmaceutical solutions to the problems that the conventional semi-solid or liquid preparations possess, enabling medicinally effective, poorly water-soluble compounds to be formulated, molded and processed, quickly and in an economically favorable manner without use of any organic solvent.

The present invention has been described in an illustrative manner, and it is to be understood the terminology used is intended to be in the nature of description rather than of limitation. Many modifications and variations of the present invention are possible in light of the above teachings. Therefore, it is to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

WHAT IS CLAIMED IS:

1. A solid dispersed preparation for poorly water-soluble drugs, prepared by dissolving or dispersing the drugs in an oil, a fatty acid or a mixture thereof, mixing the solution or dispersion in a water-soluble polyol matrix and drying the mixture.

2. The solid dispersed preparation as set forth in claim 1, wherein the solid dispersed preparation is obtained by pulverizing the dried mixture to give a dispersed powdery preparation.

3. The solid dispersed preparation as set forth in claim 1, wherein the mixture is dried in such a way that the mixture is sprayed to pharmaceutically acceptable nuclei to give a dispersed granular preparation.

4. The solid dispersed preparation as set forth in claim 1, wherein the oil, the fatty acid or the mixture thereof is used in a form of an emulsion or a micro emulsion.

5. The solid dispersed preparation as set forth in claim 1, wherein the oil is selected from the group comprising α-bisabolol, stearyl glycerrhetinate, salicylic acid, tocopheryl acetate, sodium hyaluronate, panthenol, propylene glycol and apple (*Pirus Malus*), propylene

glycol and pineapple, ivy (*Hedera halix*) extract and 1,3-B.G, peach (*Prums persica*) leaf extract, hydrolyzed soy flour, wheat (*Triticum Vulgare*) protein, birch (*Betula alba*) extract and 1,3-B.G, burdock (*Arctium majus*) extract and 1,3-B.G, liposomes, phosphatidylcholines, glyceryl stearate, captylic/capric triglyceride, cetyl octanolate, isopropyl myristate, 2-ethylene isopelagonate, di-C12-13 alkyl malate, ceteatyl octanoate, butylene glycol dicaptylate/dicaprate, isononyl isostearate, isostearyl isostearate, coco-captylate/caprate, cetyl octanoate, octyldodecyl myristate, cetyl esters, C10-30 cholesterol/lanosterol ester, hydrogenated castor oil, monoglycerides, diglycerides, triglycerides, beeswax, canauba wax, suctose distearate, PEG-8 beeswax, ceresin, ozokerite, macadamia ternifolia nut oil, hydrogenated hi-erucic acid rape seed oil, olive oil, jojoba oil, hybridsunflower (Helian thus annuus) oil, and dog rose (rosa canina) lips oil.

6. The solid dispersed preparation as set forth in claim 5, wherein the oil is selected from the group comprising mineral oils, squalene, squalane, monoglycerides, diglycerides, triglycerides, medium chain glycerides, myglyol, cremophor, hydrogenated caster oil, corn oil, perilla oil, cotton seed oil and lipid-soluble vitamins.

7. The solid dispersed preparation as set forth in claim 1, wherein the fatty acid is selected from the group comprising oleic acid, cetyl alcohol, stearyl alcohol, stearic acid, myristic acid, linoleic acid and lauric acid.

8. The solid dispersed preparation as set forth in claim 7, wherein the fatty acid is selected from the group comprising oleic acid, linoleic acid, and isopropyl myristate.

9. The solid dispersed preparation as set forth in claim 1, wherein the water-soluble polymer matrix is selected from the group comprising polyethylene glycol (PEG), carbowax and polyvinyl pyrrolidone (PVP).

10. The solid dispersed preparation as set forth in claim 1, wherein the poorly water-soluble drugs are dissolved and dispersed in the oil, fatty acid or their mixture in the presence of a surfactant.

11. The solid dispersed preparation as set forth in claim 10, wherein the surfactant is selected from the group comprising glyceryl stearate, polysorbate 60, polysorbate 80, sorbitan trioleate, sorbitan sesquioleate, sorbitan stearate, PEG-20 glyceryl isostearate, ceteth-25, PEG-60 , PEG-60 hydrogenated

castor oil, nonoxynol-15, PEG-6-decyltetradeceth-20, dimethicone copolyol, glyceryl diisostearate, ceteth-24, cetearyl alcohol, polyoxylethylene nonyphenyl ether, PEG-40 hydrogenated castor oil, cetyl dimethicone copolyol, polyglyceryl-3-methylglucose distearate, PEG-100 stearate, sorbitan isostearate, sodium lauryl glutamate, disodium cocoamphodiacetate, lauric acid diethanolamide, coconut fatty acid diethanolamide, N,N-bis-(2-hydroxy ethyl)-cocomide, and cocoamidopropyl betain.

12. The solid dispersed preparation as set forth in claim 1, wherein the water-soluble polymeric matrix is used alone or in combination with other water-soluble matrices.

13. The solid dispersed preparation as set forth in claim 12, wherein the other water-soluble matrix is selected from the group comprising gelatin, gum, carbohydrates, celluloses, polyvinyl alcohol, polyacrylic acid, inorganic compounds and their mixtures, hydroxypropylmethylcellulose acetyl succinate, shellac, zein, polyvinyl acetate phthalate, Eudragit L100, Eudragit S100, sodium arginate, and poly-L-lysine.

14. The solid dispersed preparation as set forth in claim 1, wherein the poorly water-soluble drugs are selected

from the group comprising ketoconazole, itraconazole and its derivatives, cyclosporine, cisapride, acetaminophen, aspirin, acetylsalicylic acid, indomethacin, naproxen, warfarin, papaverine, thiabendazole, miconazole, cinnarizine, doxorubicin, omeprazole, cholecalciferol, melphalan, nifedipine, digoxin, benzoic acid, tryptophan, tyrosine, phenylalanine, aztreonam, ibuprofen, phenoxymethylpenicillin, thalidomide, methyltestosterone, prochlorperazine, hydrocortisone, dideoxypurine nucleoside, vitamin D_2, sulfonamide, sulfonylurea, p-aminobenzoic acid, melatonin, benzylpenicillin, chlorambucil, diazepin, digitoxin, hydrocortisone butyrate, metronidazole benzoate, tolbutamide, prostaglandin E1(PGE_1), fludrocortisone, griseofulvin, miconazole nitrate, leukotriene B_4 antagonist, propranolol, thephylline, flubiprofen, sodium benzoate, benzoic acid, riboflavin, benzodiazepine, phenobardital, glyburide, sulfadiazine, sulfaethylthiadiazole, sodium diclofenac, aceclofenac, phyniroin, hioridazinehydrochloride, bropirimine, hydrochlorothiazide, fluconazole, acyclovir, bucillamine, ciprofluoxacin, acetyl-L-carnitine, baclofen, sodium alendronate, lovocarnitine, nimodipine or nimodifine, atenolol, provastatin sodium, lovastatin, isotretinoin, etidronate disodium, doxifluridine, fosfomycin calcium, sotepine,

epinastine hydrochloride, carvedilol, epinastine hydrochloride, carvedilol, fosinopril, trandolapril, etretinate cap, metergoline, mercaptopurine, vancomycin hydrochloride, cefixime, cefuroxim axetil, dirithramycin, and dadanosin.

15. The solid dispersed preparation as set forth in claim 13, wherein the poorly water-soluble drugs are selected from the group comprising ketoconazole, itraconazole and its derivatives, cisapride, cyclosporine, nifedipine and aceclofenac.

16. Medicines for internal use such as powders, granules, tablets and capsules, prepared using the solid dispersed preparation of claim 1.

FIG. 1

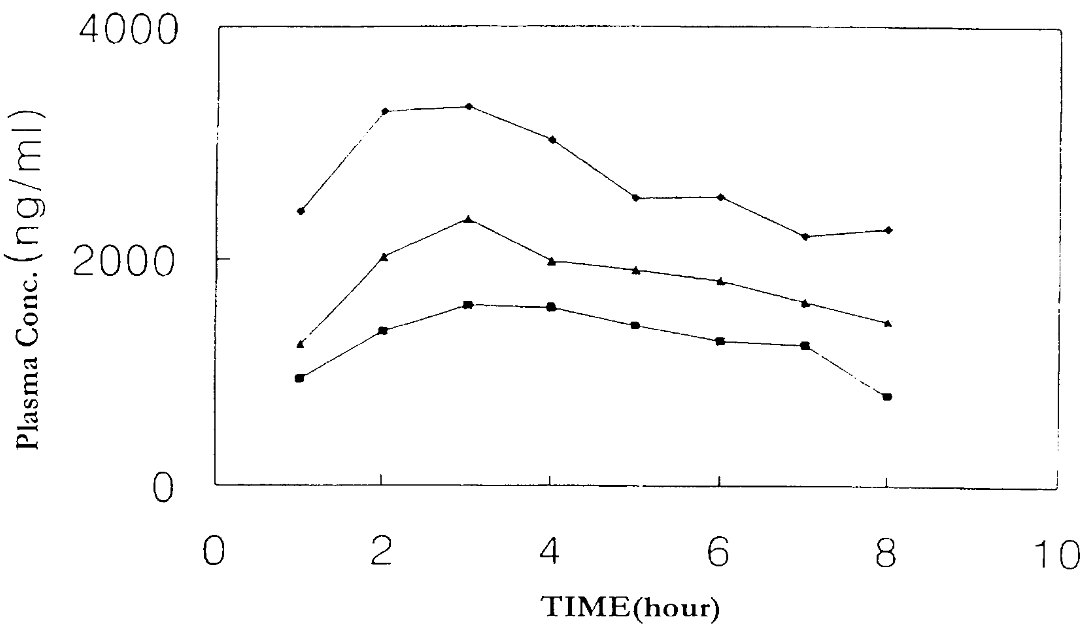

4000
2000
0
Plasma Conc. (ng/ml)
0
2
4
6
8
10
TIME(hour)

FIG. 2

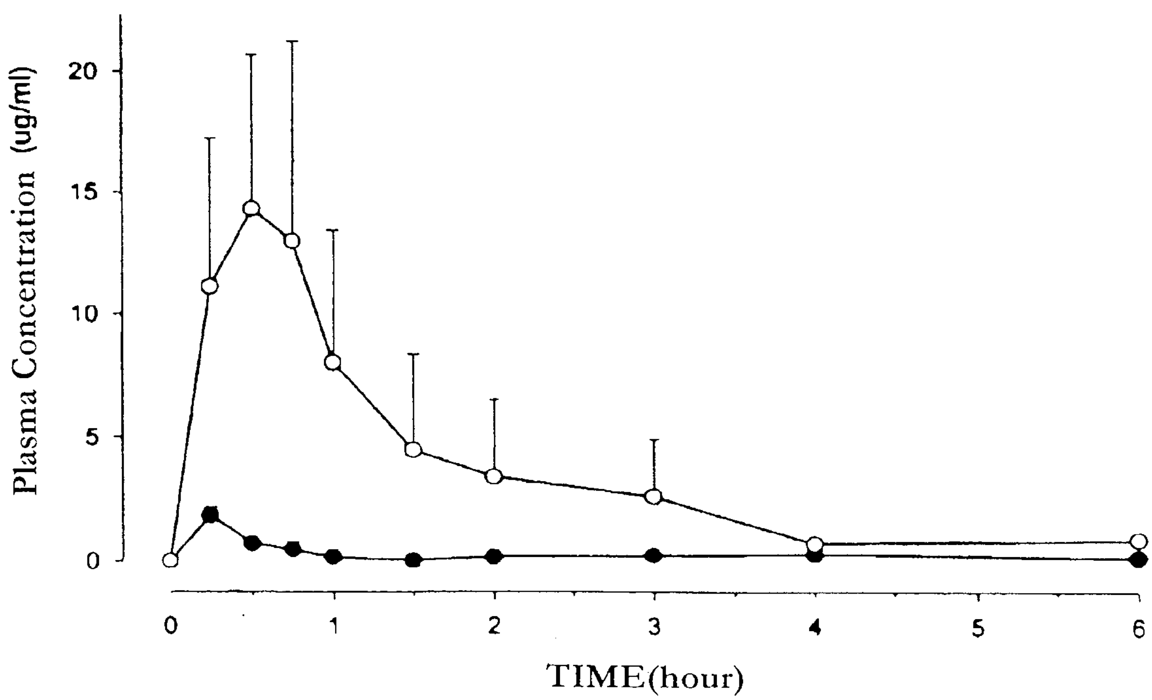
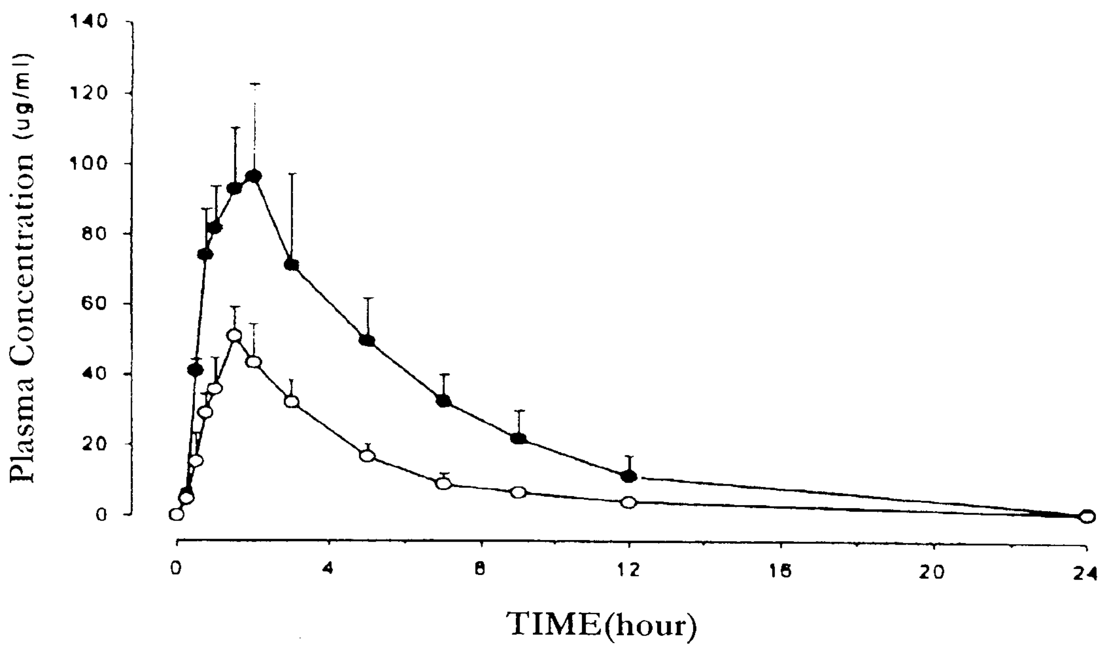
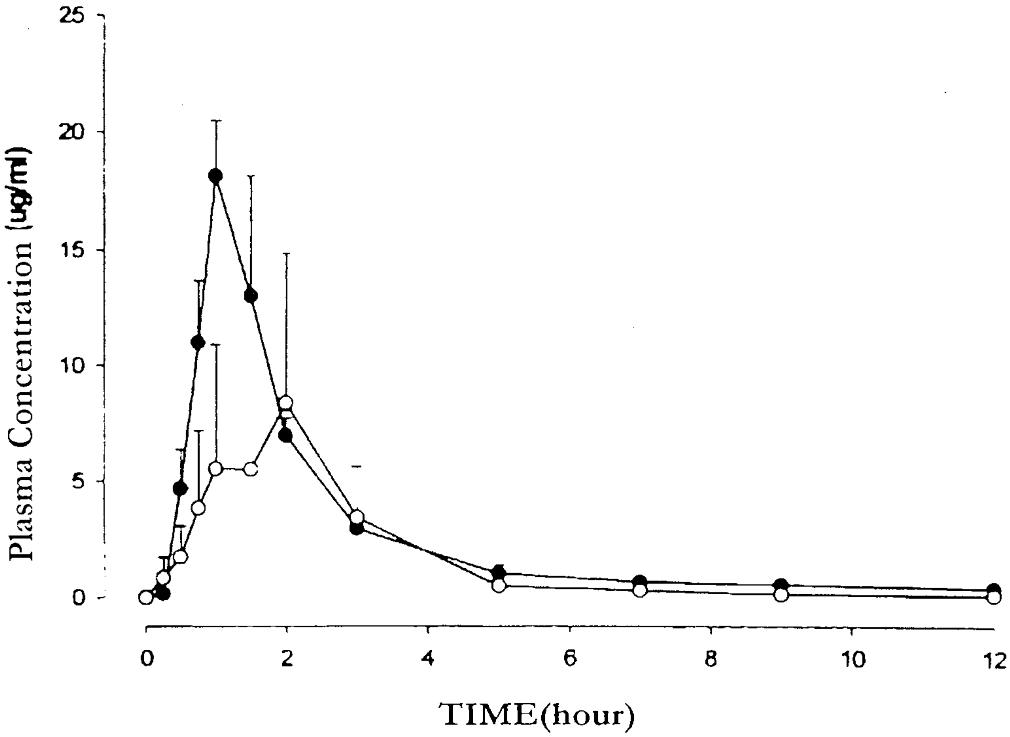

20
15
10
5
0
Plasma Concentration (ug/ml)
0
1
2
3
4
5
6
TIME(hour)

FIG. 3



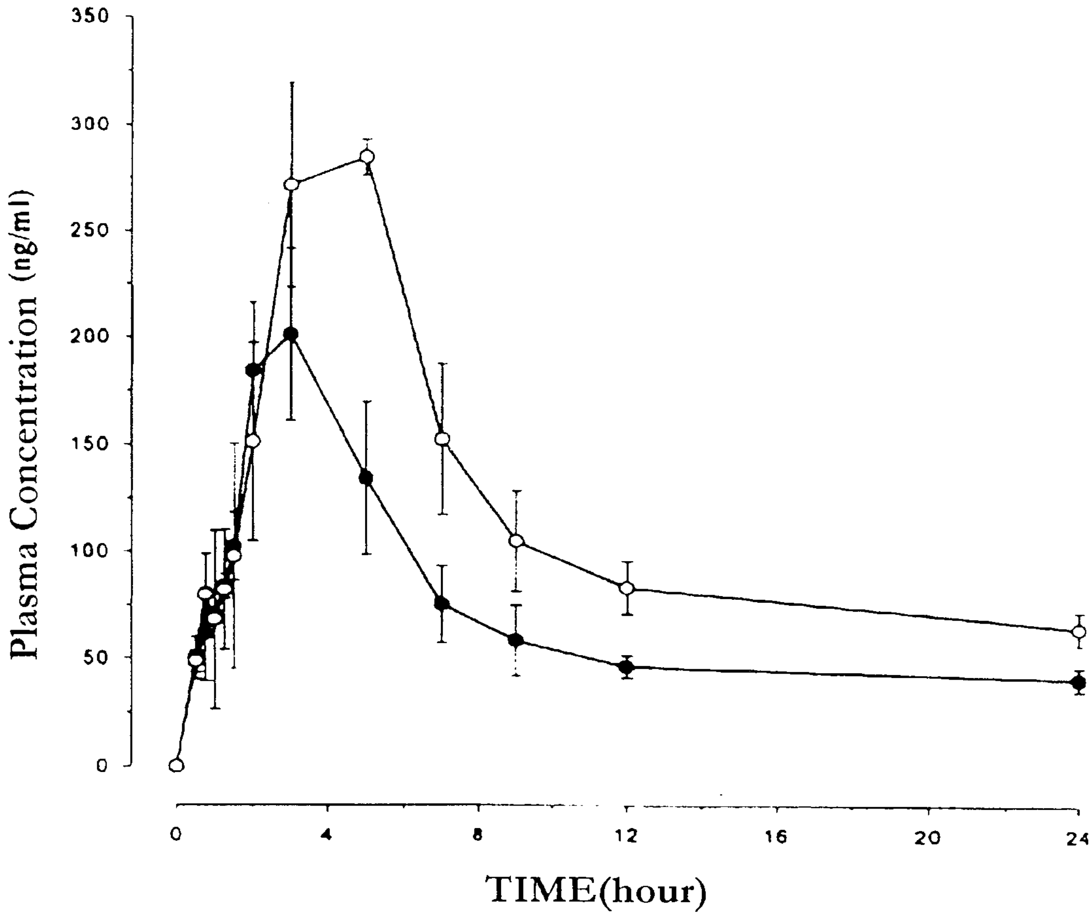
Plasma Concentration (ug/ml)
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
TIME(hour)

FIG. 4



Plasma Concentration (ug/ml)
25
20
15
10
5
0
0
2
4
6
8
10
12
TIME(hour)

FIG. 5



Plasma Concentration (ng/ml)
350
300
250
200
150
100
50
0
0
4
8
12
16
20
24
TIME(hour)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00341

A. CLASSIFICATION OF SUBJECT MATTER

IPC^6: A 61 K 9/14, A 61 K 9/16, A 61 K 9/20, A 61 K 9/48, A 61 K 31/20, A 61 K 9/107, A 61 K 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC^6: A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 October 1999 (28.10.99)	03 December 1999 (03.12.99)

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Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Krenn Telephone No. 1/53424/435

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00341

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR 99/00341

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(54) Title: CYCLOSPORIN FORMULATION

(57) Abstract: A pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, comprises a) a water-insoluble pharmaceutically active material; b) one or more propylene glycol esters of a fatty acid; c) surfactant; and either d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or e) a solid carrier. The composition is substantially free from ethanol.

CYCLOSPORIN FORMULATION

This invention relates to improved pharmaceutical compositions for the administration of water-insoluble pharmaceutically active substances especially, but not exclusively, cyclosporin.

In our European patent specification no. EP-A-0760237 there is described a pre-concentrate microemulsion composition comprising a water-insoluble pharmaceutically active material; a C_8 - C_{20} fatty acid mono-, di- or tri-glyceride from a vegetable oil or any mixture of two or more thereof; and a phospholipid and another surfactant. A stable oil-in-water microemulsion can be formed by mixing the preconcentrate composition with a hydrophilic phase. Unlike prior art microemulsion compositions, the microemulsion compositions of EP 0760237 are made by directly dissolving the active material in the oil phase and then dispersing the oil phase in the hydrophilic phase. This has certain advantages. For example, in the case of cyclosporin microemulsions, it eliminates or vastly reduces the tendency for solid microfine cyclosporin to be precipitated during use of the microemulsions, a problem encountered with many of the prior art microemulsions.

Whilst the microemulsions disclosed in EP-A-0760237 are generally very satisfactory in many ways, we have found that there is an upper limit to the bioavailability of the active material in the compositions of EP-A-0760237. We have now discovered that by judiciously alternating the components of the oil phase in the compositions of EP-A-0760237, the bioavailability of the active material can, surprisingly, be increased. The present compositions thus possess the advantages of the compositions of EP-A-0760237 together with, in addition, the advantage of increased bioavailability of the active material.

According to the present invention, there is provided a pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, which composition is substantially free from ethanol and comprises:

a) a water-insoluble pharmaceutically active material;

b) one or more propylene glycol esters of a fatty acid;

c) surfactant; and either

d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or

e) a solid carrier.

There is also provided a process for making a composition according to the invention, which process comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution either with component (d) or with component (e), and component (c) if not included earlier.

In the case of a microemulsion, the method of the invention thus comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c) but being free from hydrophilic phase, and then mixing the preconcentrate with the hydrophilic

phase, to form a stable oil-in water microemulsion, the composition being free from ethanol.

In the case of a solid composition, the method of the invention comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c), and then mixing the preconcentrate with the solid carrier, to form a solid, table composition of preconcentrate and carrier, the composition being free from ethanol.

In its broadest aspect, the present invention therefore encompasses two different formulations of the basic preconcentrate mixture. Both of these formulations possess the advantage of increased bioavailability of the active material.

Thus, in a first aspect, the invention provides a stable oil-in-water microemulsion composition wherein components (a) to (c) above have first been formed into a preconcentrate by wholly directly dissolving component (a) in component (b) optionally in the presence of component (c) (i.e. component (c) may be added later), and then mixing the preconcentrate with a hydrophilic phase. The microemulsion composition is generally liquid at room temperature and can, therefore, be advantageously provided in, for example, a soft gelatine capsule or as an oral solution such as an aqueous drink, for instance.

In a second aspect, the invention provides a stable, solid formulation comprising a blend of the basic preconcentrate mixture with a solid carrier. In this way, the preconcentrate mixture having increased bioavailability of the active material can, for example, be formulated into a free-flowing powder which, in turn can, for instance, be put into a hard gelatin capsule or compressed into a table. We generally prefer to formulate the composition of the invention in this way rather than as a microemulsion, since the solid formulation is simple to process and has excellent stability.

In the present invention, component (a) is a water insoluble pharmaceutically active material. The invention is particularly useful with the

cyclosporins, e.g. cyclosporin A. dihydrocyclosporin C, cyclosporin D and dihydrocyclosporin D. It is also useful with other water-insoluble substances such as, for example, water-insoluble peptides, or water-insoluble antimicrobial or antineoplastic substances. Examples include desmopresin, calcitonin, insulin, lenprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol, etoposide or mixtures thereof.

In the compositions of the invention, component (a) is in solution in component (b). Component (b) can be a propylene glycol ester of a fatty acid or a mixture of any two or more such esters. The fatty acids may optionally be derived from a vegetable oil and are preferably C_8 - C_{20} residues. Particular preferred compounds are propylene glycol monocaprylate (Caprgol 90) and propylene glycol monolaurate (Lauroglycol 90). We prefer to formulate the composition such that the weight ratio of component (a) to component (b) is from about 1:1 to about 1:10 but ratios outside this range can be used if desired.

These compounds, which increase the bioavailability of the active material can be used alone or in combination with one or more of the glycerides described in EP 0760237. For example, oleoyl macrogol-6 glycerides (Labrafil M 1944 CS), linoleoyl macrogol-6 glycerides (Labrafil M 2125 CS), and caprylocaproyl macrogol-8 glycerides (Labrasol) are particularly preferred compounds for use with the oils employed in the present invention.

Component (c) is a surfactant to provide the preconcentrate mixture and, where employed, the fully formed microemulsion with stability. Those skilled in the art will be aware of many surfactants which can be used, but we prefer to use polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene-sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene sorbitan monostearate. If desired, the surfactant can be mixed with a phospholipid, such as lecithin. We prefer to use a weight ratio of component (a) to surfactant of about 1:1 to about 1:50, but ratios outside this range can also be employed if desired. When a phospholipid is included in the

composition, we prefer to use a weight ratio of component (a) to phospholipid of about 1:05 to about 1:5.0, but, again, other ratios can be used.

In the case where the composition of the invention is provided as a microemulsion, component (d) is a hydrophilic phase. The preferred material is propylene glycol or diethylene glycol monoethyl ether (transcutol) but other substances can be used. Ethanol cannot be present. Water can of course also be present but it is not preferred. Despite the use of propylene glycol, component (a) remains wholly dissolved in the oil phase (component (b)).

Microemulsions are transparent due to the very small particle size of the dispersed phase, typically less than 200 nm. Such small droplets produce only weak scattering of visible light when compared with that from the coarse droplets (1 -10 nm) of normal emulsions. An essential difference between microemulsions and emulsions is that microemulsions form spontaneously and, unlike emulsions. required little mechanical work in their formulation. General reviews on microemulsions are provided by Attwood D. et al J. Colloid Interface Sci 46:249 and Kahlweit M. et al J. Colloid Interface Sci 118:436.

The microemulsions can be formed by diluting with aqueous liquid (e.g. water, fruit juice, milk etc.) to form an oil-in-water microemulsion, e.g. for oral administration. This aids in ready absorption as the surface area of the fat globules is largely increased. The role played by bile salts in the initial step of fragmentation of fat globules, essential for fat digestion, is circumvented.

The rate determining factor for the absorption of drug in the vehicle is not the enzymatic metabolism of triglycerides but rests primarily in the breakdown of the fat globules into micro particles since the enzymes (lipases) act mainly at the surface of the fat globules.

In the microemulsions of the invention, the amounts of the components, in percent by weight, are as follows:

Component	General	Usual	Preferred
Active pharmaceutical	1-12%	2.5-10%	7 -1 0%
Oil phase	20-80%	30-60%	25-40%
Surfactant	20-40%	25-60%	40-50%
Hydrophilic phase	10-60%	20-50%	25-30%

In the microemulsions, the weight percent of hydrophilic phase is generally up to about 75%, most usually from 15 to 50%, and preferably from 35 to 50%.

In the case where the composition of the present invention is provided as a blend of preconcentrate and solid carrier, component (e) is employed instead of component (d). Preferred solid carriers include colloidal silicon dioxide and polyvinyl pyrrolidone (cross Povidone) but other suitable inert solid substances can also be used, as will be clear to those skilled in the art. Typically, the solid carrier will be in the form of a dry powder. Generally, the preconcentrate mixture (comprising active material, oil and surfactant) is simply blended with the solid material such that the oily preconcentrate is absorbed by the material. Preferably, the blended mixture is provided in the form of a free-flowing powder. Such a powder can then be easily coated, for example, into a hard gelatin capsule or, alternatively, compressed into tablets, for instance. The technique of absorbing an oily phase (in this case an oily preconcentrate) on to a solid phase such as colloidal silicon dioxide followed by formulation into a final dosage form is a technique well known by those skilled in the art of formulation, so further details are considered unnecessary.

Both the microemulsion and solid compositions can consist only of the components described, or they can contain other substances. For example, in order to prevent oxidation/ rancidification of the natural oils, an antioxidant, e.g. □-to copherol can be used. Propyl gallate may be used as an alternative.

In order that the invention may be more fully understood, the following examples are given by way of illustration only.

Examples 1-3

Examples of compositions comprising a blend of preconcentrate and solid carrier are:

Example 1

Imusporin-25

Component	mg/capsule
Cyclosporin USP	25
Glyceryl Monolinoleate (Maisine 33-1)	17.25
Propylene glycol monocaprylate (Capryol 90)	17.25
Polyoxyl 35 Castor Oil NF (Cremophor EL)	50.00
Colloidal silicon dioxide	52.50
Crospovidone USP (PVP CL-M)	13.00
Net Fill Wt/cap (mg)	175.00

Example 2

Imusporin-50

Component	mg/capsule
Cyclosporin USP	50.00
Glyceryl Monolinoleate (Maisine 33-1)	34.50
Propylene glycol monocaprylate (Capryol 90)	34.50
Polyoxyl 35 Castor Oil NF (Cremophor EL)	100.00
Colloidal silicon dioxide	105.00
Crospovidone USP (PVP CL-M)	26.00
Net Fill Wt/cap (mg)	350.00

Example 3

Imusporin-100

Component	**mg/capsule**
Cyclosporin USP	100.00
Glyceryl Monolinoleate (Maisine 33-1)	69.00
Propylene glycol monocaprylate (Capryol 90)	69.00
Polyoxyl 35 Castor Oil NF (Cremophor EL)	200.00
Colloidal silicon dioxide	210.00
Crospovidone USP (PVP CL-M)	52.00
Net Fill Wt/cap (mg)	700.00

The blended preparations were made as follows:

1 Mix Maisine 35-1, Capryol 90 and Cremophor EL in a clean jacketed vessel.

2 Add Cyclosporin to the above vessel under stirring, continue stirring for about 70-75 mins. If required, heat the blend to not more than 50°C till the drug dissolves completely.

4 Cool the above blend to room temperature and strain through 150#.

5 Sift Aerosil and Crospovidone through 20# and 40# respectively. Mix in a suitable mixer.

6 Adsorb the above blend (step 4) over the mixture of Aerosil and Crospovidone.

7 Pass the powder blend of Cyclosporin through 20#.

8 Fill this blend in hard gelatin capsules or compressed with tablets.

The blends were then either fill into hard gelatin capsules or compressed into tablets.

Examples 4 - 8

Microemulsions of the invention were made of the compositions indicated, by dissolving the cyclosporin A in the oils and then forming the oil-in-water emulsions. The procedure was:

(a) dissolve the cyclosporin A in the mixture of oils with slight warming and under stirring to obtain a clear yellow liquid. Confirm the complete dissolution of the drug by microscopy.

(b) add the surfactant with stirring.

(c) add the hydrophilic phase with stirring

(d) add the alpho tocopherol and mix thoroughly.

Example 4

Preparation of microemulsion for administration in Soft Gelatin capsules:

Component	**mg/capsule**
Capryol 90	130
Castor oil	130
Polyoxyl-40 hydrogenated	400
Castor oil	-
α-tocopherol	10
Propylene glycol	200
Cyclosporin A	100

Example 5

Preparation of microemulsion for administration as oral solution:

Component	**mg/capsule**
Capryol 90	150
Maisine	125

Polysorbate-80 (Tween 80)	425
α-tocopherol	10
Transcutol	225
Cyclosporin A	100

Example 6

Preparation of microemulsion for administration as oral solution

Component	**mg/capsule**
Capryol 90	275
Polyoxyl-40 hydrogenated castor oil	425
α-tocopherol	10
Propylene glycol	225
Cyclosporin A	100

Example 7

Preparation of microemulsion for administration as oral solution:

Component	**%**
Capryol 90	130
Lauroglycol 90	130
Polysorbate 80 (Tween 80)	400
α-tocopherol	10
Propylene glycol	200
Cyclosporin A	100

Example 8

Preparation of microemulsion for administration as oral solution:

Component	mg/capsule
Capryol 90	14
Maisine	15
Polyoxyl-40 hydrogenated castor oil	45
α-tocopherol	1
Transcutol	25
Cyclosporin A	10

The oral solution which is filled into bottles can be administered using a syringe or more preferably with the aid of a metered dose pump with a dropper actuator.

The compositions described in Examples 4 to 8 were subjected to stability examinations under accelerated conditions of temperature and humidity. The solutions were stored at RT (25°C ± 2°C). Ref 40°C-80% RH and 45°C, after filling into flint glass vials.

Simultaneously with the examination of solutions prepared according to the process of the invention, the stability of the commercially available Neoral capsules containing 100mg cyclosporin A per capsule was also examined. It was observed from the above examination that the stability of solutions prepared according to the process of invention did not differ from the stability of the commercially available composition.

CLAIMS:

1. A pharmaceutical composition in the form of a preconcentrate mixed either with a liquid hydrophilic phase to form a stable oil-in-water microemulsion or with a solid carrier to form a stable, solid blend of carrier and preconcentrate, which composition is substantially free from ethanol and comprises:

a) a water-insoluble pharmaceutically active material;

b) one or more propylene glycol esters of a fatty acid;

c) surfactant; and either

d) a hydrophilic phase, wherein component (a) has been wholly directly dissolved in component (b) and component (b) is dispersed as tiny particles in component (d); or

e) a solid carrier.

2. A composition according to claim 1, which composition is a microemulsion comprising components (a), (b), (c) and (d).

3. A composition according to claim 1, which composition is a blend of said preconcentrate and said solid carrier comprising components (a), (b), (c) and (e).

4. A composition according to claim 1, 2 or 3, wherein component (a) is a cyclosporin, or another water-insoluble peptide, or a water-insoluble antimicrobial or antineoplastic substance or mixtures thereof.

5. A composition according to claim 4, wherein component (a) is cyclosporin A, dihydrocyclosporin C, cyclosporin D or dihydrocyclosporin D, or desmopresin, calcitonin, insulin, leuprolide, erythropoetin, a cephalosporin, vincristine, vinblastine, taxol or etoposide or mixtures thereof.

6. A composition according to any preceding claim, wherein component (b) is a propylene glycol ester of C_{12} to C_{18} fatty acids.

7. A composition according to any preceding claim, wherein said surfactant is polyoxyl 40 hydrogenated castor oil, polyoxyethylene-sorbitan monooleate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene-sorbitan monolaurate or polyoxyethylene-sorbitan monostearate or mixtures thereof.

8. A composition according to any preceding claim, wherein component (c) further comprises a phospholipid.

9. A composition according to any preceding claim, wherein the weight ratio of component (a) to component (b) is from 1:1 to 1:10.

10. A composition according to claim 8 or 9, wherein the weight ratio of component (a) to said phospholipid is from 1:0.5 to 1:5.0.

11. A composition according to any preceding claim, wherein the weight ratio of component (a) to said surfactant is from 1:1 to 1:5.0.

12. A composition according to any of claims 1-9 and containing component (e), wherein component (e) is colloidal silicon dioxide, polyvinyl pyrrolidone or a mixture thereof.

13. A soft gelatin capsule or oral administration fluid which comprises a composition as claimed in any of claims 1 to 11

14. A tablet or hard gelatin capsule which comprises a composition as claimed in any of claims 1 to 12 when in said solid form.

15. A process for making a composition according to claim 1, which comprises dissolving component (a) in component (b) optionally with component (c), and then mixing the resulting solution either with component (d) or with component (e) and component (c) if not included earlier.

16. A process according to claim 150, wherein a preconcentrate composition is mixed with component (d).

17. A process according to claim 15, wherein a preconcentrate composition is mixed with component (e).

18. A method of making a pharmaceutical composition according to any of claims 1 to 11, which method comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c) but being free from hydrophilic phase, and then mixing the preconcentrate with the hydrophilic phase, to form said stable oil-in-water microemulsion, the composition being free from ethanol.

19. A method of making a pharmaceutical composition according to any of claims 1-12, which method comprises first forming a preconcentrate by directly dissolving component (a) in component (b), the preconcentrate also containing component (c), and then mixing the preconcentrate with the solid carrier, to form a solid, stable composition if preconcentrated and carrier, the composition being free from ethanol.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/107 A61K9/14 A61K9/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

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X	US 5 543 393 A (KIM JUNG W ET AL) 6 August 1996 (1996-08-06) example 8 ---	1,3-6,9, 11,12,14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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(54) Title: REMOVABLE GASTRIC BAND

(57) Abstract: A removable gastric band is provided which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach. More specifically, the present removable gastric band comprises an elongated body having a first or distal zone, a second or middle zone, a third or proximal zone and a closure mechanism, wherein the closure mechanism allows the elongated body to close around a portion of the stomach, preferably the proximal tract of the stomach, wherein the closure mechanism comprises at least one aperture in the first zone and a button in the second zone, and where the button can be inserted into the aperture to close the elongated body around, and hold it to, the portion of the stomach. The removable gastric band can be easily paired with the use of a gastric electrostimulator and may be useful, therefore, for inducing forced slimming in the initial phase of treatment for morbigenous obesity. Such electrostimulation devices may either be incorporated into the removable gastric band or located at a distance from the removable gastric band.

REMOVABLE GASTRIC BAND

Related Application

This application claims priority from Italian Patent Application Number MI99A002641, filed December 7,1999.

Field of the Invention

The present invention relates to a removable gastric band which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach.

Background of the Invention

Laparoscopic banding systems are available which provide for the use of an elongated main part that is placed around the stomach and closed over the stomach so as to reduce the diameter of the stomach to be able to treat the patient's obesity. Such currently available bands, however, present some drawbacks essentially due to the difficulty of application and/or removal of the gastric band. In fact, normally, the current bands' means of closing the elongated main part are almost always hard to manipulate; moreover, their connection entails the use of additional instruments and/or devices that further complicate the application and/or later removal of the gastric band for the surgeon.

Furthermore, to be able to remove the known bands, which must necessarily be done after a more or less long time interval, it is necessary to execute an additional surgical intervention and, consequently, to administer more anesthesia to the patient. The application and/or removal of the known bands also require the application of suture stitches, in addition to another intervention and more anesthesia. In particular, the bands used today are also hard to remove because they present little resistance to tissue adhesions and lack sufficient mechanical integrity to withstand tensile forces, both of which hinder their removal unless the patient is undergoing surgery.

It is desirable, therefore, to provide an improved gastric band which is both easier to implant within the patient and, when necessary, to remove from the patient.

Summary of the Invention

The present invention provides a removable gastric band which can be used to control obesity by allowing control and/or modification of the diameter of a patient's stomach. More specifically, the present invention provides a removable gastric band comprising an elongated body having a first or distal zone, a second or middle zone, a third or proximal zone and a closure mechanism, wherein the closure mechanism allows the elongated body to close around a portion of the stomach, preferably the proximal tract of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the elongated body around, and hold it to, the portion of the stomach.

The present invention provides a removable gastric band comprising an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around a section of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

The present invention also provides a method for treatment of obesity or for reducing weight in a patient, said method comprising:

(1) positioning a removable gastric band around a section of the patient's stomach;

(2) locking the removable gastric band around the section of the patient's stomach; and

(3) adjusting the removable gastric band to control the stomach's diameter in the section of the patient's stomach,

wherein the removable gastric band comprises an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around the section of the patient's stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

The task proposed by the present invention is the realization of a removable gastric band that eliminates the above-noted drawbacks of the known gastric bands. Within the scope of this task, one important purpose of the invention is to realize a removable gastric band that can be removed without having to subject the patient to further intervention and, consequently, to additional general anesthesia.

Yet another purpose of the invention is to realize a removable gastric band that is easy to remove because it is highly resistant to adhesion to the tissue and has sufficient mechanical integrity to withstand tensile forces during removal. Yet another purpose of the invention is to realize a removable gastric band that can be applied without necessarily having to use suture stitches.

Yet another purpose of the invention is to realize a removable gastric band that can be applied and/or removed by the surgeon very simply and without having to use additional instruments or devices for that purpose. Another purpose of the invention is to realize a removable gastric band that is extremely easy to manipulate, so that it can be easily placed in and/or removed from the patient.

Brief Description of the Drawings

Figure 1 illustrates the gastric band according to the invention.

Figure 2 shows schematically the gastric band according to the invention being applied to the proximal tract of a patient's stomach;

Figure 3 shows the gastric band according to the invention applied to the proximal tract of the patient's stomach;

Figure 4 shows the gastric band according to the invention inflated so as to compress a portion of the patient's stomach;

Figure 5A shows in cross-sectional view (along line A-A in Figure 1) the inner surface of the gastric band compressing the patient's stomach before the gastric band has been inflated;

Figure 5B shows in cross-sectional view (along line A-A in Figure 1) the inner surface of the gastric band in relation to the patient's stomach after the gastric band has been inflated;

Figure 5C shows in cross-section sectional view a reinforcing member or element located within the elongated perimeter (i.e., the rib connecting the inner and outer surfaces, thereby forming an inflatable chamber or cavity) of the gastric band which reduces the tendency of the gastric band to twist around its longitudinal axis;

Figure 6A is a view of the gastric band attached to the patient's stomach with the inflation mechanism positioned to allow for inflation; and

Figure 6B is an expanded view of the inflation mechanism.

Detailed Description of the Invention

With reference to the figures described above, the removable gastric band according to the invention, indicated as a whole with reference number 1, comprises an elongated body 3 having a first or distal zone 30, a second or middle zone 32, a third or proximal zone 34, and a closure mechanism 2 for closing the elongated body 3 back upon itself so as to surround a portion, preferably the proximal tract, of the patient's stomach 4. The closure mechanism 2 preferably comprises a button 6 in the first zone 30 and a corresponding aperture 5 in the second zone 32 whereby the button 6 can fit

through the aperture 5 and fix or lock the elongated body 3 back onto to itself. Once locked into place, the gastric band 1 completely encircles and compresses a portion of the patient's stomach (see, e.g., Figures 3 and 4). Although only one aperture 5 is shown within the second zone 32, a plurality of such apertures can be provided if desired; using such a plurality of apertures allows the surgeon to more closely adjust the diameter of the encircling portion of the gastric band to the particular patient's situation and needs.

Appropriately, button 6 is suitably shaped and sized to allow it to be internally introduced into aperture 5, as well as to close, in an extremely simple but secure manner, the elongated main part 3 around stomach 4 and keep it in place. Although the button 6 and aperture 5 are preferably circular as shown in Figure 1, other shapes can be used so long as they provide the desired closing/locking action. Elongated body 3 presents at least an inner surface 7 and an outer surface 8 as more clearly shown in Figure 5A (deflated state) and Figure 5B (inflated state). Preferably, the elongated body 3 has an inflatable portion or internal cavity 9 formed by inner surface 7, outer surface 8, and ribs, welds, or closures 22 at the edges of the elongated body 3. Ribs 22 essentially form a closed space or internal cavity 9 in combination with the inner and outer surfaces 7 and 8 (see, e.g., Figure 5B). Such an inflatable member allows the elongated body 3 to be expanded when a physiological inflation medium (i.e., liquid or gas) 9 is introduced between inner surface 7 and outer surface 8. (Both the inflatable portion or internal cavity and the inflation medium, which effectively defines the size of the internal cavity, are referred to by common reference number 9 in the figures.) Preferably, button 6 is fluid-dynamically connected to inner surface 7 of elongated body 3 in such a way that, as the latter inflates, button 6 also inflates, as can be seen, for example, in Figure 3; this provides a more secure locking of the elongated body back upon itself. Preferably, the inner surface 7 is more easily expandable relative to outer surface 8 so that inflation of the elongated body 3 allows further compression, and thus more control of the compression, of the stomach. Generally, therefore, it is preferred that outer surface 8

undergoes little, if any, expansion when the physiological inflation liquid or gas 9 is introduced between inner surface 7 and outer surface 8. Compression of the stomach using the gastric band of the present invention allows for a reduction of the stomach volume as desired. The degree of compression can be modified as desired throughout the course of treatment by adding or removing inflation medium 9.

Furthermore, button 6 and aperture 5 are preferably sized relative to one another that once button 6 is passed through aperture 5 and inflated, the closure mechanism is securely activated but, once button 6 is deflated, the closure mechanism can easily be deactivated by simply pulling on one end of the gastric band (preferably by pulling on tube 20) to remove the gastric band from the abdomen. Furthermore, button 6 is preferably located outside of elongated main part 3 by a distance that can allow a substantial alignment of the first and second zones of elongated body 3, when the latter is closed around the stomach. Thus, when the elongated body 3 is inflated (and preferably button 6 is also inflated), there is no unsuitable and/or harmful superposition of two parts of the elongated body 3 that would provide an undesired enlargement at the zone where they are superposed. In other words, the inflatable portions of the gastric band do not overlap; such overlapping might result in undesirable and/or additional stomach compression in the area of overlap.

Preferably, button 6 is equipped with flap 10 that makes it easier to catch and insert the button 6 into aperture 5 using appropriate instruments. Flap 10 is appropriately made with no internal cavity and, therefore, is not inflatable. Flap 10 can be grasped quickly and simply by surgical endoscopic forceps 11 that is passed first through aperture 5 (see Figure 2). Once grasped, flap 10 and button 6 are pulled back through aperture 5 to lock the gastric band in place (see Figure 3).

As noted above, it is preferred that the button 6 expands at the same time as inner surface 7 of the elongated body 3. The expansion of button 6 should, however, be limited so that, once the gastric band 1 is locked firmly in place, the button 6 does not under go significant further expansion. For

example, the relative thicknesses of the walls of the button 6 and inner surface 7 can be controlled such that the inflation of the button will reach a definite value without expanding any further, independently of the inflation of inner surface 7 of elongated body 3. Thus, preferably the button 6 expands to a size sufficient to lock the closure mechanism 2 in place but not significantly larger.

The elongated body 3 is preferably designed so as to prevent or reduce the tendency of the elongated body 3 to rotate around its long axis as it is being placed in the proper position around the patient's stomach. For example, one or both of the ribs 22 at the edges of the elongated body 3 can contain stiffening elements 12 (see Figure 5C) which will reduce the tendency of the elongated body 3 to rotate or twist about its long axis without effecting the ability of the elongated body to fold back on itself and encircle the patient's stomach. Such stiffening elements 12 will reduce the tendency to twist as the gastric band is being positioned within the patient. Such stiffening or antirotation elements 12 will tend to stabilize the prosthesis and make the insertion easier. The ribs 22 at the edges of the elongated body 3 are preferably gently curved so as not to create problems either at the time of the implant or during removal by pulling of elongated body 3 from the outside; in other words, the ribs, as well as other portions of the gastric band, preferably present smooth and gently curved surfaces to allow the gastric band to slide easily around organs during implantation and removal.

The gastric band preferably has an inflation mechanism 15 comprising a reservoir 16 for receiving the inflation medium, preferably a physiological liquid or gas, for inflating both elongated body 3 and button 6. Preferably, the reservoir 16 has several concentric layers 17 to allow it to be pierced, for example with needle 18, without the inflation medium 9 being able to escape from the perforation. Preferably, reservoir 16 is constructed with multiple layers of material (preferably elastomeric or plastic materials) that, when pricked with needle 18, allows the hole to be made without skewing or leakage between the different layers 17. Such skewing or leakage would generally be mainly noticeable or chiefly accentuated during the expansion of

reservoir 16 when the inflation medium 9 would tend to leak. The external layer of reservoir 16, preferably constructed of biocompatible materials, is generally thicker than the other, internal layers and can even be rigid, since it preferably remains adjacent to the abdominal wall, more preferably within the subcutis, and presents such dimensions as to permit easy introduction through a surgical laparoscopic trocar. By maintaining the reservoir 16 near the abdominal wall, the compression of the stomach can more easily be modified as desired by addition or removal of the inflation medium 9. In some instances, it may be desired for the reservoir 16 to remain outside the abdominal wall.

The elongated body 3 can be inflated using the inflation medium introduced into the reservoir 16 using, for example, a syringe 18 as shown in Figures 6A and 6B. The elongated body is inflated until the desired degree of compression of the stomach occurs. The inflation of the gastric band is generally performed under the control of the endoscopist, who can observe, preferably using an endoscope from inside the stomach, the diameter of the gastric restriction induced by the inflation of the gastric band, particularly by inner surface 7. Preferably, essentially the entire length of the gastric band 3 encircling the stomach can be inflated using the inflation medium 9.

Reservoir 16 is preferably located in the third or proximal zone 34 of elongated body 3 and is connected to the second or middle zone 32 containing aperture 5 is present via tube 20. The length of tube 20 can be varied as needed for particular patients; preferably, tube 20 does not significantly expand when inflation medium 9 is added to the gastric band. In operation, the reservoir 16 is preferably not secured and remains in the subcutis of the abdominal wall. It may be located, using, for example, feel or ultrasound, for introduction of the inflation medium in order to inflate or deflate the gastric band. Using such a technique, the diameter of the gastric constriction provided by the gastric band can be modified or adjusted as desired. Preferably, reservoir 16 has a flap 21 which can be grasped using appropriate instruments to assist in the inflation or deflation operation.

Preferably, both the main portion of the elongated body 3 and the tube 20 have stiffening or antirotation elements 12 within the ribs 22 as shown in Figure 5C. For example, the stiffening elements 12 could be a thin steel, other metal, or other type wire that is fused into the plastic material of the rib 22. Such a stiffening element 12 reduces the tendency of the gastric band to rotate about its long axis before the closure mechanism is activated. Additionally, it makes the gastric band considerably stronger (i.e., acting as a reinforcing element); this added strength may be especially important when the gastric band is removed from the patient by pulling on the proximal end 34 from the outside. The stiffening element 12, when formed using a steel or other suitable metal wire, can also be observed using X-rays, thereby determining the exact position of the band inside the patient's abdomen. Preferably, such stiffening element 12 extends essentially the entire length of the elongated body 3 (i.e., through the first, second, and third zones, including tube 20).

When it is desired to remove the gastric band from the abdomen, it is generally preferred to remove at least a portion of the inflation medium 9 so that the closure mechanism 2 can more easily be disengaged. A significant portion of the inflation medium 9 can be removed using, for example, a syringe using essentially the same procedures as used for the initial inflation process. Alternatively, tube 20 can be cut using cutting device 11a to separate reservoir 16, as represented in Figure 3, to release inflation medium 9. Preferably, at least a portion of inflation medium 9 is removed prior to cutting tube 20 so as to minimize release of inflation medium 9 into the abdominal cavity. For this purpose, under local anesthesia, a small cutaneous incision is made in the abdominal wall to access reservoir 16, at which time tube 20 is cut and the reservoir 16 is removed from the abdominal cavity. After the closure mechanism 2 is disengaged, the gastric band 3 can be removed from the abdominal cavity by pulling on the tube 20 through the small cutaneous incision.

Preferably, the limit of expandability of inner surface 7 is linked to the limit of compressibility of the gastric walls and the two ends of the elongated

body must be blunted enough to allow sliding between the patient's tissues in the phase of removal from the abdomen. In the removal phase, the gastric band will behave as an abdominal drainage tube. Preferably, the materials of construction and the surface smoothness are such that they will impede the production of fibrotic scar adhesions, as normally occurs with drainage tubes or prostheses of silicone materials. Such a smooth surface helps to prevent tissue adhesion to the gastric band. Thus, once deflated and unbuttoned, the gastric band can be removed easily by pulling on one end through a small incision. Preferably, the gastric band will have sufficient strength to withstand the forces associated with removal by this technique.

The gastric band of the present invention can be easily paired with the use of a gastric electrostimulator 100 and may be useful, therefore, for inducing forced slimming in the initial phase of treatment for morbigenous obesity. The electrostimulator 100 may be incorporated into the design of the gastric band as shown in Figure 1 (i.e., attached to the inner surface 7) such that the electrostimulator 100 is in contact with the stomach when the gastric band is properly positioned. Alternatively, it may be separately implanted elsewhere within the abdominal cavity as shown in Figure 2 (e.g., attached to the antrum). If incorporated into the gastric band design, the electrostimulator 100 is implanted at the same time as, and held in place by, the gastric band, thereby eliminating separate attachment of the electrostimulator 100. In such a unitary design, however, the electrostimulator 100 must be removed at the same time as the gastric band. If such an electrostimulator 100 is separately placed at a distance from the gastric band, it may remain within the abdominal cavity after removal of the gastric band. The selection of the preferred location of such an electrostimulator 100 relative to the gastric band will depend largely on the particular patient's requirements and planed treatment regime. Both the electrostimulator 100 and the gastric band are preferably installed and/or removed at the same time, thereby reducing the extent of surgical intervention and anesthesia.

Conventional electrostimulation devices 100 may be used in the practice of this invention in combination with the gastric band 3. Such

devices include, for example, those described in U.S. Patent 5,423,872 (June 3, 1995) (an implantable gastric electrical stimulator at the antrum area of the stomach which generates sequential electrical pulses to stimulate the entire stomach, thereby artificially altering the natural gastric motility to prevent emptying or to slow down food transit through the stomach); U.S. Patent 5,690,691 (November 25, 1997) (a portable or implantable gastric pacemaker employing a number of electrodes along the greater curvature of the stomach for delivering phased electrical stimulation at different locations to accelerate or attenuate peristaltic movement in the gastrointestinal tract); U.S. Patent 5,836,994 (November 17, 1998) (an implantable gastric stimulator which incorporates direct sensing of the intrinsic gastric electrical activity by one or more sensors of predetermined frequency bandwidth for application or cessation of stimulation based on the amount of sensed activity); U.S. Patent 5,861,014 (January 19, 1999) (an implantable gastric stimulator for sensing abnormal electrical activity of the gastrointestinal tract so as to provide electrical stimulation for a preset time period or for the duration of the abnormal electrical activity to treat gastric rhythm abnormalities); U.S.Patent 6,041,258 (March 21, 2000) (electrostimulation device with improved handle for laparoscopic surgery); U.S. Patent Application Serial Number 09/640,201(filed August 16, 2000) (eletrostimulation device attachable to enteric or endo-abdominal tissue or viscera which is resistance to detachment); PCT Application Serial Number PCT/US00/09910 (filed April 14, 2000; Attorney Docket No. 3581/006 PCT) entitled “Gastric Stimulator Apparatus and Method for Installing” based on United States Provisional Application Serial Numbers 60/129,198 and 60/129,199 (both filed April 14, 1999); PCT Application Serial Number PCT/US00/10154 (filed April 14, 2000; Attorney Docket No. 3581/004 PCT) entitled “Gastric Stimulator Apparatus and Method for Use” based on United States Provisional Application Serial Numbers 60/129,209 (filed April 14, 1999) and 60/466,387 (filed December 17, 1999); and U.S. Provisional Patent Application Serial Number 60/235,660 (filed September 26, 2000) entitled “Method and Apparatus for Intentional Impairment of Gastric Motility and/or

Efficiency by Triggered Electrical Stimulation of the Gastric Tract with Respect to the Intrinsic Gastric Electrical Activity." All of these patents, patent applications, provisional patent applications, and/or publications are hereby incorporated by reference.

Moreover, the gastric band of the invention is of great clinical interest, especially in relation to problems inherent to prolonged permanence in the abdomen, that is, intragastric decubitus, perforation, strangulation, and the like. In practice it has been confirmed that the removable gastric band according to the invention is particularly advantageous because it can be removed without having to perform an additional surgical intervention and additional anesthesia on the patient, thanks especially to its qualities of resistance to pulling.

The invention thus conceived is susceptible to numerous modifications and variations, all falling within the scope of the inventive concept; furthermore, all of the details can be substituted with technically equivalent elements. In practice, other materials and dimensions can be used, depending on the demands and on the state of the technique.

Claims

That which is claimed is:

1. A removable gastric band comprising an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around a section of the stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

2. The removable gastric band of claim 1, wherein at least the portion of the elongated body encircling the section of the stomach comprises an essentially planar inner surface, an essentially planar outer surface, and ribs running along the elongated body and connecting the inner and outer surfaces to form an internal cavity, such that the cavity can be inflated whereby the inner surface can controllably compress the section of the stomach.

3. The removable gastric band of claim 2, wherein the button is fluid-dynamically connected to the cavity and is inflatable, whereby the elongated body can be more securely closed around the section of the stomach when the cavity is inflated.

4. The removable gastric band of claim 3, wherein the button is located outside of the elongated body by a distance to allow substantial alignment of the first and second zones of the elongated body when closed around said stomach.

5. The removable gastric band of claim 3, wherein the button has a flap for catching and easy introduction into the aperture.

6. The removable gastric band of claim 5, wherein the ribs have reinforcing elements to reduce the tendency of the elongated body to rotate around its long axis.

7. The removable gastric band of claim 6, wherein the second and third zones are connected by a tube and the third zone has a reservoir for receiving an inflation medium and wherein the reservoir is fluid-dynamically connected to the cavity, whereby the cavity can be inflated or deflated by adding or removing, respectively, inflation medium from the reservoir.

8. The removable gastric band of claim 7, wherein the reservoir comprises a sphere having a plurality of concentric layers to allow the reservoir to be pierced with a needle without allowing the inflation medium to escape.

9. The removable gastric band of claim 8, wherein the reinforcing elements are radiopaque.

10. The removable gastric band of claim 7, wherein the reservoir has a flap for easy holding.

11. The removable gastric band of claim 8, wherein the reservoir has a flap for easy holding.

12. The removable gastric band of claim 2, wherein essentially planar inner surface of the portion of the elongated body encircling the section of the stomach has an electrostimulator that contacts the stomach when the gastric band is in placed around the stomach.

13. The removable gastric band of claim 7, wherein the essentially planar inner surface of the portion of the elongated body encircling the section of the stomach has an electrostimulator that contacts the stomach when the gastric band is in place around the stomach.

14. A method for treatment of obesity in a patient, said method comprising:

(1) positioning a removable gastric band around a section of the patient's stomach;

(2) locking the removable gastric band around the section of the patient's stomach; and

(3) adjusting the removable gastric band to control the stomach's diameter in the section of the patient's stomach,

wherein the removable gastric band comprises an elongated body having a first zone, a second zone, a third zone, and a closure mechanism, wherein the closure mechanism allows a portion of the elongated body to close around the section of the patient's stomach, wherein the closure mechanism comprises a button in the first zone and at least one aperture in the second zone, such that the button can be inserted into the aperture to close the portion of the elongated body around, and hold it to, the section of the stomach, and wherein the portion of the elongated body is essentially planar in cross section.

15. The method of claim 14, wherein at least the portion of the elongated body encircling the section of the stomach comprises an essentially planar inner surface, an essentially planar outer surface, and ribs running along the elongated body and connecting the inner and outer surfaces to form an internal cavity, such that the cavity can be inflated whereby the inner surface can controllably compress the section of the stomach.

16. The method of claim 15, wherein the button is fluid-dynamically connected to the cavity and is inflatable, whereby the elongated body can be

more securely closed around the section of the stomach when the cavity is inflated.

17. The removable gastric band of claim 16, wherein the button is located outside of the elongated body by a distance to allow substantial alignment of the first and second zones of the elongated body when closed around said stomach.

18. The method of claim 16, wherein the button has a flap for catching and easy introduction into the aperture.

19. The method of claim 18, wherein the ribs have reinforcing elements to reduce the tendency of the elongated body to rotate around its long axis.

20. The method of claim 19, wherein the second and third zones are connected by a tube and the third zone has a reservoir for receiving an inflation medium and wherein the reservoir is fluid-dynamically connected to the cavity, whereby the cavity can be inflated or deflated by adding or removing, respectively, inflation medium from the reservoir.

21. The method of claim 20, wherein the reservoir comprises a sphere having a plurality of concentric layers to allow the reservoir to be pierced with a needle without allowing the inflation medium to escape.

22. The method of claim 21, wherein the reinforcing elements are radiopaque.

23. The method of claim 20, wherein the reservoir has a flap for easy holding.

24. The method of claim 21, wherein the reservoir has a flap for easy holding.

25. The method of claim 15 further comprising implanting an electrostimulator near or adjacent to the patient's stomach and providing electrostimulation to the patient's stomach in combination with the gastric band.

26. The method of claim 25, wherein the electrostimulator is located on the essentially planar inner surface of the portion of the elongated body encircling the section of the patient's stomach such that the electrostimulator contacts the patient's stomach when the gastric band is in place around the patient's stomach.

27. The method of claim 25, wherein the electrostimulator is located separately from the gastric band.

28. The method of claim 20 further comprising implanting an electrostimulator near or adjacent to the patient's stomach and providing electrostimulation to the patient's stomach in combination with the gastric band.

29. The method of claim 28, wherein the electrostimulator is located on the essentially planar inner surface of the portion of the elongated body encircling the section of the patient's stomach such that the electrostimulator contacts the patient's stomach when the gastric band is in place around the patient's stomach.

30. The method of claim 28, wherein the electrostimulator is located separately from the gastric band.

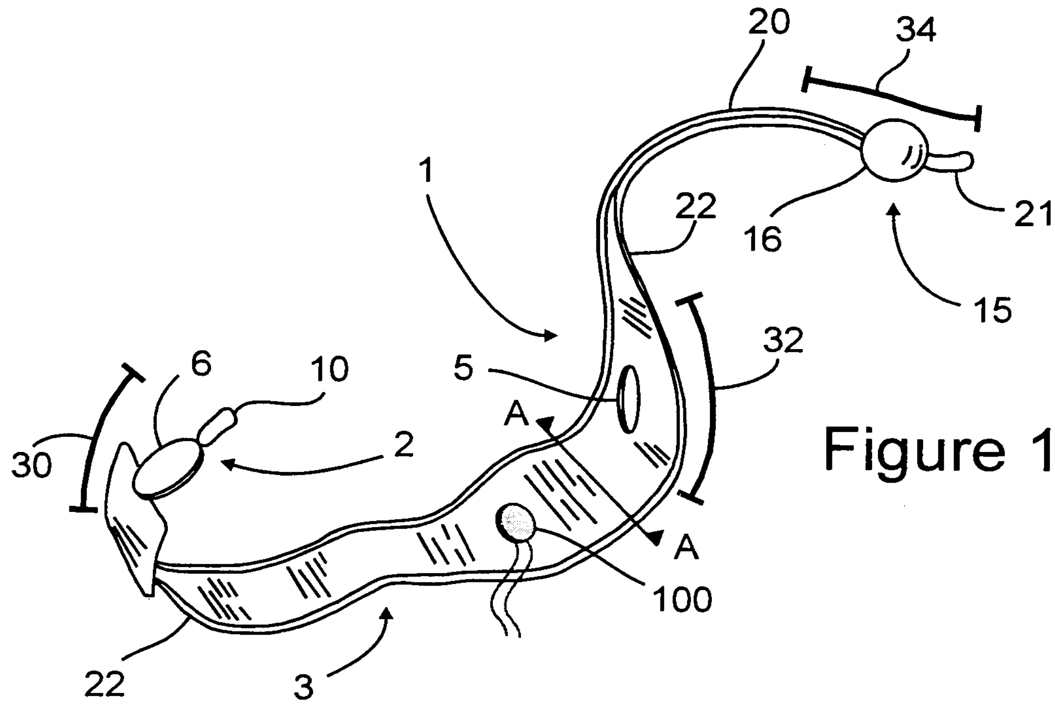


Figure 1

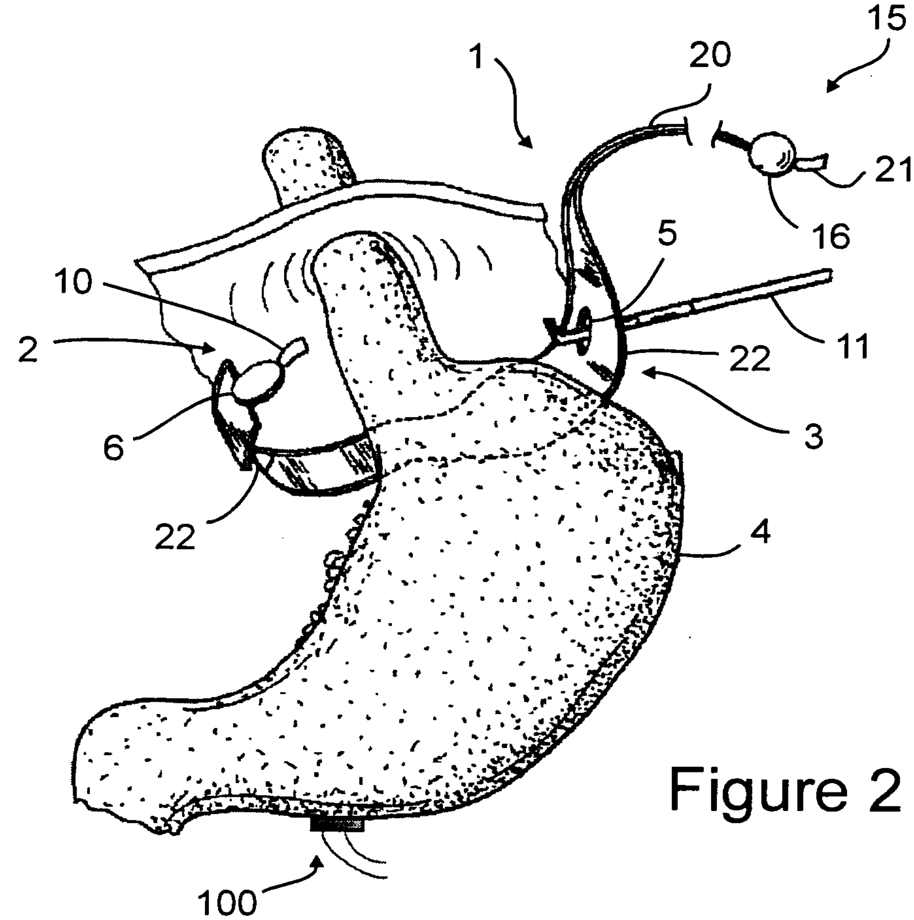
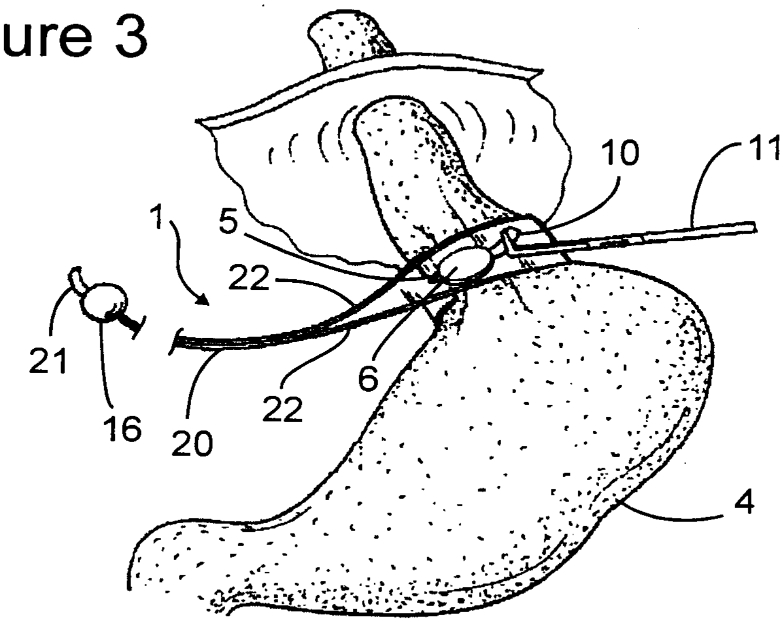


Figure 2

Figure 3



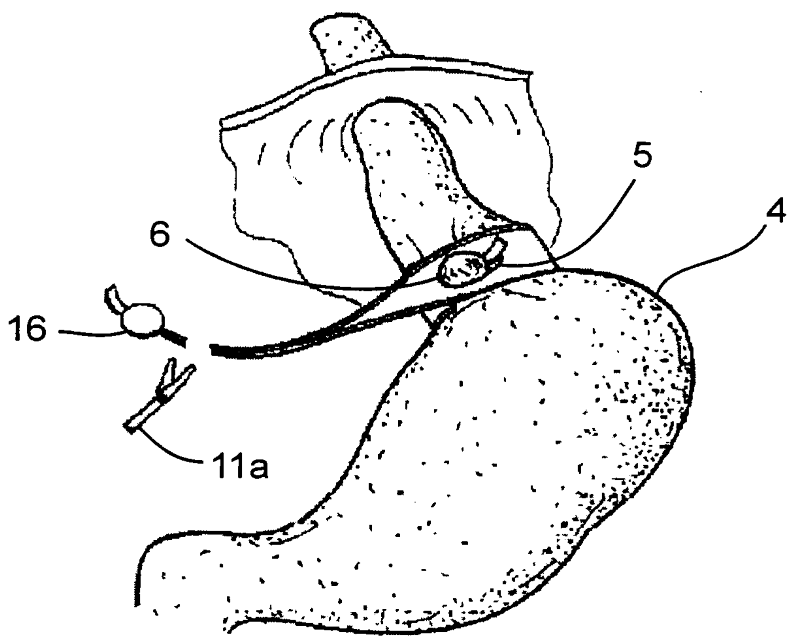


Figure 4

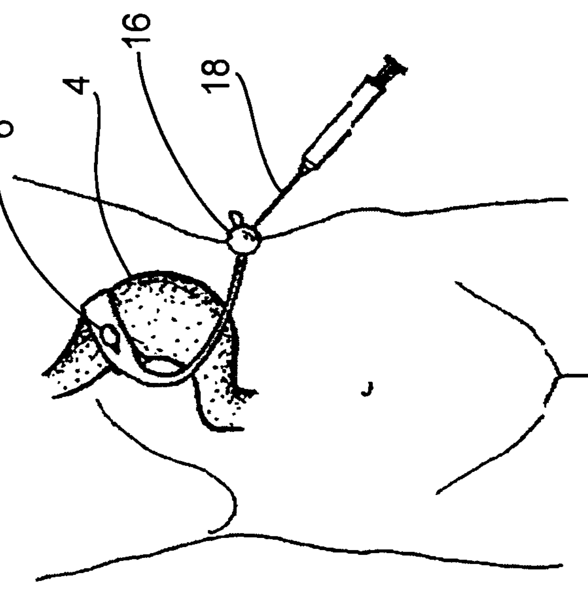


Figure 6A

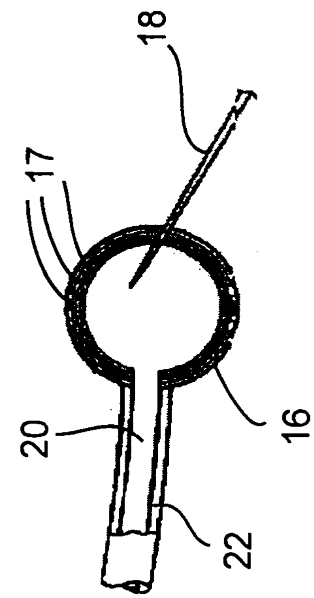


Figure 6B

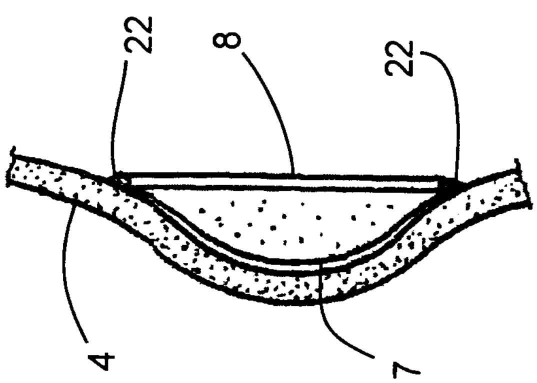


Figure 5B

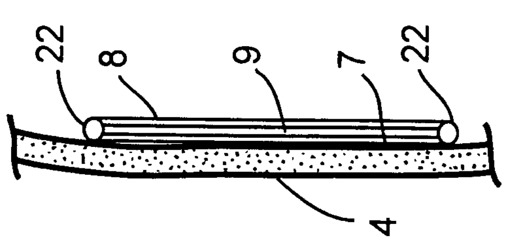


Figure 5A

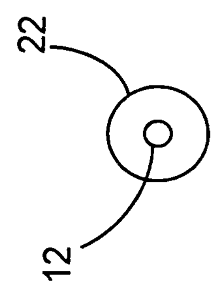


Figure 5C

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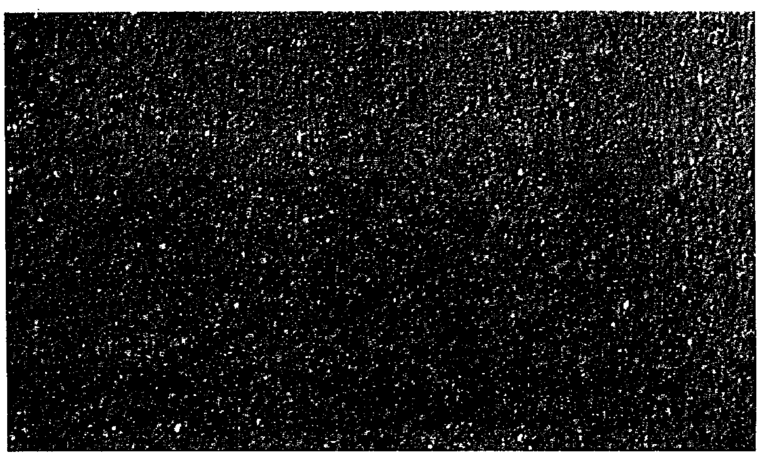
[Fortsetzung auf der nächsten Seite]

(54) Title: DISPERSIONS FOR FORMULATING SLIGHTLY OR POORLY SOLUBLE ACTIVE INGREDIENTS

(54) Bezeichnung: DISPERSIONEN ZUR FORMULIERUNG WENIG ODER SCHWER LÖSLICHER WIRKSTOFFE



1 Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/mL Amphotericin B aus Beispiel 19.

1 OPTICAL MICROSCOPE PHOTO OF THE EMULSION CONTAINING 5mg/mL AMPHOTERICIN B, FROM EXAMPLE 19

(57) Abstract: The invention relates to a dispersion, comprising an oily phase and an aqueous phase in the form of an O/W emulsion or a W/O emulsion, at least one active ingredient which is slightly or poorly soluble in the oily and the aqueous phases, in addition to optionally one or more emulsifiers and/or stabilisers. The dispersion is devoid of toxicologically questionable organic solvents and contains a dissolved quantity of said active ingredient that is higher than the additive quantity obtained by its maximum solubility in both the oily and the aqueous phase of the emulsion.

[Fortsetzung auf der nächsten Seite]

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Erklärung gemäß Regel 4.17:
— *Erfindererklärung (Regel 4.17 Ziffer iv) nur für US*

Veröffentlicht:
— *ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts*

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(57) Zusammenfassung: Die Erfindung betrifft eine Dispersion, die eine ölige Phase und eine wässrige Phase in Form einer O/W-Emulsion oder W/O-Emulsion, mindestens einen in der öligen und der wässrigen Phase wenig oder schwer löslichen Wirkstoff sowie gegebenenfalls einen oder mehrere Emulgator(en) und/oder Stabilisator(en) umfasst, wobei die Dispersion frei von toxikologisch bedenklichen organischen Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der öligen und der wässrigen Phase der Emulsion ergibt.

Dispersionen zur Formulierung wenig oder schwer löslicher Wirkstoffe

Die Erfindung betrifft Dispersionen, die eine ölige Phase, eine wäßrige Phase und in diesen beiden Phasen wenig löslichen, schwer löslichen bis zu unlöslichen Arzneimittelwirkstoff umfassen.

Wirkstoffe mit geringer Löslichkeit haben sehr oft das Problem einer unzureichenden Bioverfügbarkeit. Der generelle Lösungsansatz für dieses Problem ist die Erhöhung der Löslichkeit dieser Wirkstoffe. Beispiele hierfür sind die Lösungsvermittlung über Solubilisation, Bildung von Einschlußverbindungen (z. B. mit Cyclodextrinen) sowie die Verwendung von Lösungsmittelgemischen (K. H. Bauer, K.-H. Frömming, C. Führer, Pharmazeutische Technologie, Georg Thieme Verlag Stuttgart, 1991). Für viele Wirkstoffe führt dies jedoch nicht zu einer ausreichenden Erhöhung der Löslichkeit, insbesondere wenn Wirkstoffe gleichzeitig schwerlöslich in wäßrigen Medien und gleichzeitig schwerlöslich in organischen Medien sind. Hier scheiden z. B. Lösungsmittelgemische als Lösung für das Problem aus. Alternativ können gering wasserlösliche Wirkstoffe in Ölen gelöst werden, eine O/W-Emulsion hergestellt und diese dann oral oder parenteral (in der Regel i.v.) appliziert werden. Sehr viele Wirkstoffe, insbesondere Wirkstoffe mit gleichzeitig geringer Löslichkeit in wäßrigen und organischen Medien, sind jedoch nicht ausreichend in Ölen löslich. Nicht ausreichend bedeutet, daß aufgrund zu geringer Löslichkeit bei erforderlicher Dosis das zu applizierende Volumen der Emulsion zu groß wird.

In Wasser und in Ölen gering lösliche Wirkstoffe wie Amphotericin B können trotzdem in Emulsionen eingearbeitet werden (Seki et al. US 5 534 502). Um dies zu erreichen müssen jedoch zusätzliche organische Lösungsmittel eingesetzt werden. Diese Lösungsmittel müssen dann in Zwischenschritten der Emulsionsherstellung oder dem Produkt wieder entzogen werden (Davis, Washington, EP 0 296 845 A1) wobei jedoch ein gewisser Restlösungsmittelgehalt im Produkt verbleibt. Zusätzlich ist diese Herstellung sehr zeitaufwendig und kostenintensiv, so daß Produkte basierend auf dieser Technologie praktisch auf dem Markt nicht vertreten sind. Eine alternative Methode ist die Einlagerung von derartigen Substanzen wie Amphotericin B in die Phospholipid-Doppelmembran von Liposomen, Handelsprodukt ist beispielsweise Ambisome® (Janknegt et al., Liposomal and lipid formulations of amphotericin B, Clin. Pharmacokinet., 23, 279-291 [1992]). Nachteilig ist aber auch hier die sehr teure Herstellung, so daß es in der Regel nur in Notfällen eingesetzt wird, wenn eine andere Behandlung nicht zum Ziel führt bzw. nur bei Patienten eingesetzt wird, die finanziell in der Lage sind, die Behandlung zu bezahlen. Somit besteht eindeutig ein Bedarf an einer kostengünstigen Formulierung, die gleichzeitig möglichst einfach herzustellen ist, im Gegensatz zu Liposomen lagerstabil ist und eine Lyophilisation nicht erfordert sowie nicht von Restlösungsmitteln belastet ist.

Der vorliegenden Erfindung liegt daher die Aufgabe zugrunde, eine Dispersion zur Verfügung zu stellen, die einen wenig, schwer oder sogar bisher unlöslichen Wirkstoff in einer bisher nicht möglichen Menge gelöst enthält, wobei gleichzeitig die oben beschriebenen Nachteile der Verwendung zusätzlicher zur Formulierung bisher notwendiger organischer Lösungsmittel entfällt.

Gegenstand der vorliegenden Erfindung ist daher eine Dispersion auf der Basis einer O/W-Emulsion oder einer W/O-Emulsion beladen mit Wirkstoff, der in Wasser und gleichzeitig auch in Ölen wenig löslich oder schwer löslich bis hin zu unlöslich ist, wobei diese Dispersion frei von toxikologisch bedenklichen organischen

Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der Wasser- und der Ölphase der Emulsion ergibt.

Insbesondere ist die erfindungsgemäß gelöste Menge um den Faktor 2, bevorzugter 5, noch bevorzugter 10 oder noch größer als die additive Menge.

Die "additive Menge" wird durch Auflösen der maximalen Wirkstoffmenge in den separaten öligen und wäßrigen Phasen (bei ansonsten identischen Lösebedingungen) entsprechend den Anteilen in der Dispersion ermittelt (Sättigungskonzentrationen), wobei keine weiteren zusätzlichen organischen Lösungsmittel zum Einsatz kommen. Die erfindungsgemäße Dispersion enthält zusätzlich zu der additiven Menge ein überadditive Menge an gelöstem Wirkstoff.

Ein wichtiges erfindungsgemäßes Merkmal ist, daß bei gleicher Zusammensetzung hochenergetisch homogenisiert wird, im Vergleich zu niederenergetischem Dispergieren (Schütteln oder Blattrührer).

Die Herstellung der erfindungsgemäßen Dispersion erfolgt insbesondere unter Ausschluß von toxikologisch bedenklichen organischen Lösungsmitteln wie z.B. Methylenchlorid und Ethanol. Die Wirkstoffe werden unter Umgehung eines Zwischenschrittes direkt aus der festen Substanz in die Emulsion eingearbeitet.

Detaillierte Beschreibung der Erfindung

Generell ist es anerkannter Stand der Wissenschaft, daß die Moleküle eines schwerlöslichen oder gering löslichen Wirkstoffes aus dem festen Aggregatzustand (Pulver) über mindestens einen Zwischenschritt (z. B. molekulardisperse Verteilung in einem Lösungsmittel) in eine Emulsion als Trägersystem eingearbeitet werden müssen. Die Erfahrung zeigt, daß bei in Wasser und Öl gleichzeitig sehr gering löslichen Substanzen es nicht genügt,

eine Emulsion mit Kristallen des Wirkstoffes zu versetzen. So führt die teilweise praktizierte Zumischung von Amphotericin B-Lösung (Lösungsmittelgemisch) zu einer handelsüblichen O/W-Emulsion wie Intralipid oder Lipofundin zur Präzipitation des Wirkstoffes, es entstehen Amphotericin B-Kristalle, die sedimentieren und sich nicht mehr in der Emulsion auflösen.

Überraschender Weise wurde jedoch nun gefunden, daß die Herstellung eines Emulsionssystems mit gelöstem Wirkstoff auch direkt aus dem festen Aggregatzustand des Wirkstoffes möglich ist. Zur Herstellung der erfindungsgemäßen Dispersion wird der Wirkstoff in partikulärer Form der Wasserphase oder der Ölphase zugesetzt und anschließend alle Komponenten einem höher energetischen oder hochenergetischen Prozeß wie z. B. der Homogenisation, insbesondere der Hochdruckhomogenisation unterzogen. Der hochenergetische Prozeß der Hochdruckhomogenisation führt dazu, daß der Wirkstoff in die Emulsion molekulardispers eingearbeitet wird und keine Wirkstoffkristalle mehr im Polarisationsmikroskop detektierbar sind. Die erhaltenen Emulsionen sind überraschender Weise ähnlich stabil wie Systeme, die unter Einsatz von organischen Lösungsmitteln erzeugt worden sind.

Eine sehr einfache Art der Einarbeitung der Wirkstoffkristalle ist die Verreibung des Wirkstoffes mit einer handelsüblichen O/W-Emulsion (z. B. Lipofundin, Intralipid). Nach Anreiben befindet sich der Wirkstoff primär in der Wasserphase, es ist ein disperses System entstanden, das als innere Phase gleichzeitig Öltropfen und Wirkstoff-Kristalle enthält. Dieses disperse System wird dann homogenisiert oder hochdruckhomogenisiert (z. B. 1.500 bar und 5 – 20 Homogenisationszyklen). Es wird eine feindisperse Emulsion erhalten (Beispiel 1), in der am Ende des Homogenisationsprozesses keine Wirkstoff-Kristalle mehr nachweisbar sind. Die Kristalle haben sich daher nahezu vollständig oder vollständig aufgelöst, d.h. daß sich im Lichtmikroskop selbst bei 1000 facher Vergrößerung in 2 von 3 Feldern nicht mehr als 10

Kristalle, vorzugsweise nicht mehr als 5 Kristalle und insbesondere nicht mehr als 1 Kristall nachweisen lassen/läßt.

Falls es gewünscht ist, kann der Wirkstoff jedoch auch in einer solchen Menge eingesetzt werden, daß am Ende des Homogenisationsprozesses neben dem gelösten Anteil des Wirkstoffs noch ein Anteil des Wirkstoffs in ungelöster kristalliner Form vorliegt, der ein Depot bildet.

Alternativ kann eine wäßrige Suspension des Wirkstoffes mit einer O/W-Emulsion gemischt werden. Es handelt sich wieder um ein disperses System mit einer dispergierten Phase aus Öltropfen und Wirkstoff-Kristallen. Dieses wird ebenfalls einem höher oder hochenergetischem Prozeß wie der Hochdruckhomogenisation unterzogen. Die Zumischung einer wäßrigen Suspension des Wirkstoffes eignet sich insbesondere dann, wenn die Wirkstoffkonzentration relativ gering ist. Zusätzlich kann die wäßrige Suspension des Wirkstoffes vor der Zumischung einem in den Lehrbüchern beschriebenem Mahlprozeß unterzogen werden, z. B. Naßmahlung mit einer Kolloidmühle, einer Kugelmühle oder einer Perlmühle oder durch Hochdruckhomogenisation vorzerkleinert werden.

Generell ist es günstig, den Wirkstoff in der Form sehr feiner Kristalle zu verwenden, d. h. in mikronisierter Form mit einer Teilchengröße im Bereich von ca. 0,1 µm - 25 µm (Kolloidmühle, Gasstrahlmühle).

Alternativ kann der Wirkstoff auch im Öl dispergiert werden. Das Öl mit den Wirkstoff-Kristallen wird dann in der Wasserphase dispergiert, wobei das dafür notwendige Tensid entweder der Wasserphase zugesetzt wird oder in der Ölphase gelöst wird bzw. jeweils dispergiert wird. Im Falle von Lecithin kann das Lecithin im Wasser dispergiert werden oder in der Ölphase unter leichtem Erwärmen gelöst werden.

Bei Einarbeitung der Wirkstoff-Kristalle in die Ölphase kann dies ohne Zusatz eines Tensids erfolgen. Das Tensid, z. B. Lecithin, wird anschließend zugesetzt. Alternativ können auch die Wirkstoff-Kristalle in eine Ölphase eingearbeitet werden, die bereits Tensid enthält.

Nach Einarbeitung der Wirkstoff-Kristalle in das Öl wird die Ölphase in Wasser dispergiert (z. B. mit einem hochtourigen Rührer) und die erhaltene Rohemulsion anschließend hochdruckhomogenisiert. Auch hier ist es günstig, die Wirkstoff-Kristalle möglichst klein einzusetzen. Zur weiteren Zerkleinerung der in die Ölphase eingearbeiteten Wirkstoff-Kristalle kann diese ölige Suspension vor dem Herstellen der Rohemulsion zunächst einer Mahlung unterzogen werden. Die Wirkstoff-Kristalle in der Ölphase werden durch diese Naßmahlung weiter zerkleinert, teilweise bis in den Nanometerbereich. Übliche Verfahren der Naßmahlung, die eingesetzt werden können, sind z. B. die Kolloidmühle und die Hochdruckhomogenisation der Ölphase. Generell ist die Kavitation einer wäßrigen Phase das anerkannte Prinzip der Zerkleinerung bei der Hochdruckhomogenisation, d. h. die Anwesenheit von Wasser ist zur Kavitation erforderlich. Öle mit einem zu Wasser extrem geringen Dampfdruck sind zur Kavitation nicht fähig. Trotzdem wurde überraschender Weise gefunden, daß eine zur Herstellung des neuen Trägersystems ausreichende Zerkleinerung auftritt.

Charakteristisch für die erfindungsgemäße Dispersion ist, daß der in der Emulsion eingearbeitete Wirkstoff in höherer Menge gelöst vorliegt als es sich additiv aus seiner maximalen Löslichkeit in der Wasser- und Ölphase der Emulsion ergibt und gleichzeitig zur Herstellung keine toxikologisch bedenklichen organischen Lösungsmittel eingesetzt wurden. Zu solchen toxikologisch bedenklichen organischen Lösungsmitteln gehören insbesondere Chloroform, Methylenchlorid, längerkettige Alkohole wie Hexanol und Octanol, aber auch ethanol in höheren Konzentrationen.

In der Regel handelt es sich bei den erfindungsgemäßen Wirkstoffen um Wirkstoffe, die wenig löslich (1 Teil löst sich in 30-100 Teilen Lösungsmittel) oder schwer löslich (1 Teil in 100-1000 Teilen Lösungsmittel), insbesondere aber sehr schwer löslich (1 Teil löst sich in 1.000 bis 10.000 Teilen Lösungsmittel) oder sogar unlöslich sind (> 10.000 Teile Lösungsmittel).

So beträgt die Löslichkeit von Amphotericin B in Wasser weniger als 0,001% (< 0,01 mg/ml) bei pH 6-7, das heißt dem pH-Wert der Emulsion. Die Löslichkeit von Amphotericin ist zwar höher bei pH 2 und pH 11 (0,1 mg/ml), jedoch sind diese Lösungen nicht intravenös applizierbar.

Die Löslichkeit von Amphotericin in Sojaöl (Long Chain Triglycerides – LCT) und in Miglyol 812 (Medium Chain Triglycerides – MCT), den Standardölen für die meisten auf dem Markt befindlichen Emulsionen zur parenteralen Infusion ist kleiner als 0,0001 mg/ml.

40g Emulsion aus Beispiel 1 bestehen zu 20% aus Öl (8g) und ca. 80% aus Wasser (32g). Somit lassen sich aufgrund der Löslichkeiten 8 x 0,0001 mg/ml plus 32 x 0,01 mg, d.h. insgesamt 0,3208 mg Amphotericin in 40g Emulsionsbestandteilen Öl und Wasser auflösen, d.h. 0,008 mg/ml. In der vorliegen erfindungsgemäßen Emulsion konnten 0,2 mg/ml Emulsion eingearbeitet werden (Beispiel 1) ohne daß mikroskopisch Kristalle von ungelöstem Arzneistoff detektierbar waren (Beispiel 12). Auch höhere Konzentration wie 1 mg/ml Emulsion konnten eingearbeitet werden (Beispiel 2), mit Laserdiffraktometrie waren keine der zur Herstellung eingesetzten Arzneistoffpartikel mehr detektierbar (Beispiel 11).

Bei einer gewünschten Dosis von z.B. 100 mg Amphotericin B ergibt sich bei den erfindungsgemäßen Dispersionen mit 1 bzw. 0,2 mg/ml Emulsion ein intravenös zu applizierendes Volumen von 100 bis 500 ml Emulsion. Somit werden mit der erfindungsgemäßen Emulsion

wenig lösliche und schwer lösliche Wirkstoffe erst in einem ausreichend kleinen Applikationsvolumen bei verträglichen pH-Werten applizierbar.

Gelöster Wirkstoff ist schnell verfügbar. Zur Erzeugung eines Depots kann mehr Wirkstoff in die Dispersion eingearbeitet werden als sich darin löst, d h. man erzeugt Kristalle, die als Depot wirken. Die Löslichkeit in Wasser und Ölphase betragen z.B. für Amphotericin B 0,008 mg/ml, die erfindungsgemäße Emulsion löst ohne detektierbare Kristalle z.B. 0,2 mg/ml (Beispiel 1). Arbeitet man 5 mg/ml Dispersion ein, so ist die Löslichkeit überschritten (übersättigtes System). Nach Hochdruckhomogenisation erhält man zusätzlich zum gelösten Wirkstoff noch hochfeine Arzneistoffkriställchen (Beispiel 15).

Die durch Mischung von Arzneistoff (Beispiel 15) oder einer Arzneistoffsuspension (analog Beispiel 6) mit einer Emulsion und anschließende Homogenisation hergestellten heterogenen, übersättigten Dispersionen sind dadurch gekennzeichnet, daß separat nebeneinander Öltropfen und hochfeine Kriställchen existieren, d.h. die Kristalle sind primär außerhalb der Öltropfen.

Die Bestimmung der Partikelgröße erfolgt mit Lichtmikroskopie unter Ermittlung der Anzahlverteilung. Alternativ erfolgt die Bestimmung mit Laserdiffraktometrie (Gerät: Coulter LS 230, Coulter Electronics, Krefeld, Germany), wobei die erhaltene Volumenverteilung in die Anzahlverteilung umgerechnet wird.

Sind in der Dispersion bei hoher Beladung mit Wirkstoff neben den Emulsionstropfen noch Arzneistoffkristalle vorhanden, so sind direkt nach der Herstellung mindestens 90%, bevorzugt 95% der Anzahl der Wirkstoffkristalle in der Anzahlverteilung kleiner als 5 µm. Bei Anwendung von hohen Drücken (z.B. 1000 bar) und einer ausreichenden Anzahl an Homogenisationszyklen erhält man hochdisperse Systeme. In Abhängigkeit von Druck und Zyklenzahl erhält man Dispersionen mit mindestens 90%, teilweise 95% und

insbesondere 99% der Anzahl der Kristalle in der Anzahlverteilung kleiner als 1 µm.

Oben wurde die in situ Erzeugung des Wirkstoff-Depots aus Kriställchen durch Herstellung der erfindungsgemäßen Dispersion mit einer Wirkstoffmenge oberhalb der Sättigungslöslichkeit des Systems beschrieben. Alternativ kann auch eine erfindungsgemäße Dispersion mit ausschließlich gelöstem Wirkstoff hergestellt werden, der man nachträglich Wirkstoffkristalle definierter Größe zumischt, z.B. mikronisierter Wirkstoff.

Zur Herstellung der erfindungsgemäßen Dispersion können handelsübliche O/W-Emulsionen eingesetzt werden (z.B. Lipofundin, Intralipid, Lipovenös, Abbolipid, Deltalipid und Salvilipid) oder es wird eine Emulsion aus Ölphase, Emulgator / Stabilisator und äußerer Phase (z.B. Wasser) hergestellt.

Beispiele für Bestandteile der Ölphase der Emulsionen sind: Sojaöl, Safloröl (Distelöl), langkettige Triglyceride (LCT), mittelkettige Triglyceride (MCT) wie z.B. Miglyole, Fischöle und Öle mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierte Partialglyceride wie Stesolid, einzeln oder in Mischungen.

Zur Stabilisierung der Dispersionen können Emulgatoren und Stabilisatoren eingesetzt werden. Diese sind gegebenenfalls bereits in der zur Herstellung der erfindungsgemäßen Dispersion eingesetzten Emulsion enthalten, Zusatz weiterer Emulgatoren und Stabilisatoren bei der Herstellung der Dispersion kann vorteilhaft sein.

Beispiele für Emulgatoren sind z.B. Ei-Lecithin, Soja-Lecithin, Phospholipide aus Ei oder Soja, Tween 80, Natriumglykocholat und Natriumlaurylsulfat (SDS). Alternativ kann Stabilisierung durch Zusatz von Substanzen erfolgen die über andere Mechanismen als Emulgatoren stabilitätserhöhend wirken, z.B. über sterische

Stabilisierung oder Erhöhung der Zetapotentials. Solche Stabilisatoren sind z.B. Block-Copolymere wie z.B. Poloxamere (z.B. Poloxamer 188 und 407) und Poloxamine (z.B. Poloxamine 908), Polyvinylpyrrolidon (PVP), Polyvinylalkohol (PVA), Gelatine, Polysaccharide wie Hyaluronsäure und Chitosan und ihre Derivate, Polyacrylsäure und ihre Derivate, Polycarbophil, Cellulosederivate (Methyl-, Hydroxypropyl- und Carboxymethylcellulose), Zuckerester wie Saccharosemonostearat und Antiflokkulatien wie Natriumcitrat. Emulgatoren und Stabilisatoren können einzeln oder in Mischungen verwendet werden. Typische Konzentrationen sind 0,1% bis 20%, insbesondere 0,5% bis 10%.

Als wäßrige äußere Phase der zur Herstellung der erfindungsgemäßen Dispersion eingesetzten O/W-Emulsion können dienen: Wasser, Mischungen von Wasser mit anderen wassermischbaren organischen Flüssigkeiten, flüssige Polyethylenglykole (PEG, insbesondere PEG 400 und 600).

Die wäßrige äußere Phase kann auch Zusätze enthalten, z.B. Elektrolyte, Nichtelektrolyte (z.B. Glycerol, Glucose, Mannit, Xylit zur Isotonisierung), Gelbildner wie Cellulosederivate und Polysaccharide wie Xanthan und Alginate (z.B. zur Viskositätserhöhung).

Für die topische Applikation können der Dispersion Penetrationsverstärker (z.B. Azone, Laurinsäure) und für die Applikation zum Gastrointestinaltrakt Absorptionsverstärker (z.B. Gallensäuren, Lysophospholipide) zugesetzt werden.

Wirkstoffe zur Einarbeitung in die Emulsion sind neben Amphotericin B z.B. Ciclosporin, Buparvaquon und Atovaquon. Weitere Wirkstoffe sind Hormone (z.B. Estradiol), Antioestrogene und Kortikoide (z.B. Prednicarbat).

Die Applikation der Emulsion kann auf verschiedenen Wegen erfolgen, z.B. parenteral aber auch oral oder topisch. Bei

parenteraler Applikation sind alle gängigen Wege möglich, z.B. intravenös, intra- und subkutan, intramuskulär, intraartikulär, intraperitoneal etc.

Topische Emulsionen mit Ciclosporin können die Wirkstoffpenetration in die Haut verbessern aufgrund des hohen gelösten Anteils an Arzneistoff (erhöhter Konzentrationsgradient). Orale Applikation der Ciclosporin-Emulsion kann die Bioverfügbarkeit erhöhen da im Gegensatz zu mikronisiertem Ciclosporin ein erhöhter gelöster Anteil vorliegt.

Die Bioverfügbarkeit von oral appliziertem Amphotericin B ist aufgrund seiner geringen Löslichkeit nahezu Null. Orale Applikation der Amphotericin-Emulsion kann aufgrund des erhöhten gelösten Anteils ebenfalls die Bioverfügbarkeit erhöhen.

Die erfindungsgemäßen Emulsionen (z.B. mit Buparvaquon und Atovaquon) können nach intravenöser Injektion auch durch Anlagerung einer Targeting-Einheit (z.B. Apolipoprotein E in Kombination mit Apolipoprotein AI und AIV) für eine gewebsspezifische Arzneistoffapplikation eingesetzt werden (Targeting zum Gehirn). Erreger lokalisieren bei bestimmten Erkrankungen des monozytären phagocytierenden Systems (MPS) auch im Gehirn und sind bisher schwer einer Therapie zugänglich (z.B. Leishmaniosen, Toxoplasmose).

Die oben beschriebenen Systeme sind vom Typ O/W, d. h. Öltropfen sind dispergiert in einer Wasserphase. Es ist jedoch auch möglich, Dispersionen auf der Basis von W/O-Emulsionen zu produzieren. Ein grundsätzlicher Vorteil ist, daß die äußere Ölphase als eine Diffusionsbarriere fungiert und die Freigabe des Arzneistoffes verzögert. Derartige Dispersionen können nicht intravenös appliziert werden, aber sie können zum Beispiel intramuskulär oder subkutan als Depotformulierung injiziert werden. Applikation dieser W/O-Systeme am Auge erhöht die Verweilzeit aufgrund der erhöhten Viskosität und gleichzeitig

wird eine verlängerte Arzneistofffreisetzung erreicht. Bei topischer Applikation auf die Haut hat die Ölphase einen okklusiven Effekt, der zu einer erhöhten Arzneistoffpenetration führt. Daher besitzen diese W/O-Typ-Systeme einen Vorteil für spezielle Anwendungen. Bevorzugte Form der Erfindung ist jedoch die Dispersion auf der Basis des O/W-Typs.

Bei Öl-in-Wasser Emulsionen ist die Dispersion dadurch gekennzeichnet, daß sie 5 bis 99,5 Gew.-% wäßrige Phase, vorzugsweise 10 bis 95 Gew.-% wäßrige Phase, besonders bevorzugt 60 bis 95 Gew.-% wäßrige Phase und speziell 70-95% wäßrige Phase, jeweils bezogen auf die Gesamtmenge der Dispersion, enthält.

Bei Wasser-in-Öl Emulsionen ist die Dispersion dadurch gekennzeichnet, daß sie aus 5 bis 30 Gew.-% wäßriger Phase, vorzugsweise 10 bis 25 Gew.-% wäßriger Phase, besonders bevorzugt 10 bis 20 Gew.-% wäßriger Phase, jeweils bezogen auf die Gesamtmenge der Dispersion, enthält.

Die Bestandteile der Ölphase der Emulsionen sind – wie oben ausgeführt - insbesondere ausgewählt aus der Gruppe bestehend aus Sojaöl, Safloröl (Distelöl), langkettigen Triglyceriden (LCT), mittelkettigen Triglyceriden (MCT), wie z. B. Miglyole, Fischölen und Ölen mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierten Partialglyceriden, wie in Stesolid®, einzeln oder in Mischungen. Die mittelkettigen Triglyceride enthalten vorzugsweise wenigstens 90 % Triglyceride der Capryl-Säure (C8) und der Caprin-Säure (C10). Als Ölphase sind im Rahmen der Erfindung Gemische aus Sojaöl und MCT, vorzugsweise im Gewichtsverhältnis 5:1 bis 1:5, besonders bevorzugt zwischen 2:1 und 1:2 oder 1:1 geeignet.

Die Fettphase der erfundenen Dispersion kann aus Ölen bestehen, d.h. die Lipide sind bei einer Raumtemperatur von 20°C flüssig. Es besteht weiterhin die Möglichkeit, daß diese Öle mit Lipiden gemischt werden, die bei einer Raumtemperatur von 20°C fest sind.

Die Mischungsanteile von Öl zu festem Lipid können von 99 + 1 bis 1 + 99 (Gewichtsanteile) variieren. Bevorzugt sind Mischungen, die mindestens 10 Teile flüssiges Öl enthalten, speziell mindestens 30 Teile flüssiges Öl und insbesondere mindestens 50 Anteile flüssiges Öl.

In speziellen Fällen kann die Lipidphase der Dispersion zu 100% Lipide enthalten, die bei einer Raumtemperatur von 20°C fest sind. Schmelzen die Lipide nahe der Raumtemperatur, können Dispersionen erhalten werden, deren Lipidtröpfchen sich in einem Zustand einer "Unterkühlten Schmelze" befinden. Liegen sehr hochschmelzende Lipide vor, können – ungeachtet der durch die Thomson-Gleichung beschriebenen Schmelzpunktionsdepression – die Partikel der Dispersion aushärten. Die Thomson-Gleichung beschreibt, daß der Schmelzpunkt von Lipiden gegenüber ihrer "bulk"-Ware stark herabgesetzt wird, wenn diese in sehr feinen Partikeln auskristallisieren (z. B. Nanopartikel oder Partikel in einem Größenbereich von wenigen Mikrometern) (Hunter, R.J., Foundations of colloid science, Vol. 1, Oxford University Press, Oxford, 1986).

Beispiele für bei Raumtemperatur feste Lipide sind, Karnaubawachs, Hydroxyoctacosanylhydroxystearat, Chinesisches Wachs, Cetylpalmitat, Bienenwachs und ähnliche Wachse. Weitere Beispiele für feste Lipide beinhalten C_{20-40} Di- und Triglyceride, mit gesättigten und ungesättigten Fettsäuren, C_{20-40} Fettalkohole, C_{20-40} Fettamine und ihre Verbindungen, sowie Sterole.

Als Lipide zur Herstellung von Mischungen aus flüssigen und festen Lipiden sind geeignet: Natürliche oder synthetische Triglyceride bzw. Mischungen derselben, Monoglyceride und Diglyceride, alleine oder Mischungen derselben oder mit z. B. Triglyceriden, selbst-emulgierende modifizierte Lipide, natürliche und synthetische Wachse, Fettalkohole, einschließlich ihrer Ester und Ether und Mischungen derselben. Besonders geeignet sind synthetische Monoglyceride, Diglyceride und Triglyceride als

individuelle Substanzen oder als Mischung (z. B. Hartfett), Imwitor 900, Triglyceride (z. B. Glyceroltrilaurat, Glyceroltrimyristat, Glyceroltripalmitat, Glyceroltristearat und Glyceroltribehenat) und Wachse wie z. B. Cetylpalmitat, Karnaubawachs und weißes Wachs (DAB). Außerdem Kohlenwasserstoffe, wie z. B. Hartparaffin.

Die Tropfengröße der Öltropfen (O/W-Typ) oder Wassertropfen (W/O-Typ) in der Dispersion ist größer als 100 nm (bestimmt mit Photonenkorrelationsspektroskopie – PCS). Das empfohlene obere Größenlimit für die Tropfen ist 10 µm, anderenfalls kommt es zum Aufrahmen aufgrund der Flotation der Tropfen, was zu physikalischer Instabilität führt (Tropfenkoaleszenz). Um Flotation zu minimieren, sollte die Größe kleiner als 5 µm sein, vorzugsweise unterhalb von 1 µm (PCS-Durchmesser), was zu den sogenannten physikalisch "autostabilen" Dispersionen führt. Die optimale Stabilität wurde gefunden im Größenbereich ähnlich zu parenteralen Fettemulsionen mit PCS-Durchmessern von 200 nm bis 500 nm.

Der Gehalt an Stabilisatoren in parenteralen Zubereitungen sollte so niedrig wie möglich gehalten werden, um Toxizität und Störungen des Metabolismus zu minimieren. Von Lecithin-haltigen Emulsionen zur parenteralen Ernährung ist es bekannt, daß eine zu hohe Zuführung von Lecithin metabolische Störungen bewirken kann, typische Tagesvolumina appliziert sind hier z. B. 500 ml Emulsion und mehr. Dies führte zu der Entwicklung der Lecithin-reduzierten Emulsionen, d. h. man reduzierte den Lecithingehalt von 1,2% weiter auf nur 0,6% Lecithin. Einige Systeme zur Applikation von schwerlöslichen Arzneistoffen verwenden einen relativ hohen Emulgatorgehalt (z. B. Solubilisierung mit Tensiden, SEDDS – self-emulsifying drug delivery systems basierend auf der Solubilisation von Öl mit hohen Tensidkonzentrationen). Eine spezielle Eigenschaft der vorliegenden Erfindung ist, daß sie die Tensidbelastung minimiert. Eine typische Zusammensetzung des O/W-Types der erfindungsgemäßen Dispersion ist: 20 g Öl, 1,2 g Lecithin, 0,1 g Arzneistoff und 78,3 g

Wasser. Dies bedeutet, daß die 21,2 g produzierter Öltropfen aus 20 g Ölphase (= 94,3%) und 1,2 g Stabilisator (= 5,7%) bestehen.

Weitere Beispiele für Emulgatoren sind neben Lecithinen die Polyethoxysorbitanester (Tween®-Typen), wie beispielsweise Laurate (Tween 20/21), Palmitate (Tween 40), Stearate (Tween 60/61), Tristearate (Tween 65), Oleate (Tween 80/81), oder Trioleate (Tween 85), Natriumglycocholat und Natriumlaurylsulfat (SDS) sowie die Sorbitanfettsäureester (Span® -Typen). Besonders bevorzugt ist Tween 80.

Bevorzugt werden weiterhin Tenside, Emulgatoren und Stabilisatoren eingesetzt, die für die Anwendung am und im Menschen zugelassen sind (z.B. Hilfsstoffe mit dem GRAS-Status).

Speziell für die Dispersionen vom Typ W/O werden die typischen Wasser-in-Öl-Tenside zur Stabilisierung benutzt, manchmal in Mischungen, auch in Mischungen mit O/W-Emulgatoren. Beispiele hierfür sind die Fettalkohole, Ethylenglykolmonostearat, Glycerolmonostearat, Sorbitanfettsäureester (Span®-Serie, z. B. Span 20-, Span 40-, Span 60- und Span 80-Serie, speziell Span 85), Ether von Fettalkoholen mit Polyethylenglykol (PEG) (z. B. Brij®-Serie), Ester von Fettsäuren mit PEG (z. B. Myrj®-Serie).

Im allgemeinen werden wieder Tenside und Stabilisatoren mit einem anerkannten Status bevorzugt, z. B. GRAS-Substanzen (Generally Regarded As Safe — Food Additives — GRAS substances, Food Drug Cosmetic Law Reports, Chicago (1994), Food Additive Database der FDA, Internet: www.fda.gov, 1999).

Im Fall daß die erfindungsgemäßen Dispersionen — zusätzlich zu den Öltropfen — noch Partikel von ungelöstem Wirkstoff enthalten, sollte die Partikelgröße so klein wie möglich sein, zum Beispiel zwecks Erhalt der physikalischen Stabilität und zur Vermeidung von Sedimentation. Zusätzlich, im Fall der intravenösen Applikation, sollten die Partikel klein genug sein, um Kapillar-

blockade zu vermeiden. Die kleinsten Blutkapillaren sind ungefähr 5-6 µm im Durchmesser. Daher sollte der Partikeldurchmesser 90% unterhalb von 5 µm sein, vorzugsweise auch der Durchmesser 95% und insbesondere der Durchmesser 100% sollte unterhalb 5 µm sein (gemessen mit Laserdiffraktometrie nach Abtrennung der Partikel von der Dispersion durch Zentrifugation, Volumenverteilungsdaten). Es ist noch günstiger, wenn diese Durchmesser alle unterhalb von 3 µm sind, da dann eine Sicherheitsdistanz zur Größe der kleinsten Kapillaren vorhanden ist.

Am vorteilhaftesten ist eine Partikelgröße des ungelösten Arzneistoffes unterhalb von 1000 nm (mittlere Partikelgröße gemessen mit Photonenkorrelationsspektroskopie). Diese Größe ist weit weg von den 5-6 µm der kleinsten Kapillardurchmesser und schließt gleichzeitig jegliche Sedimentationseffekte aus (diese Partikelgröße sedimentiert nicht relativ unabhängig von der Dichte des Arzneistoffes). Im Fall, daß eine schnellere Auflösung der Arzneistoffkristalle nach Applikation der Dispersion notwendig ist, sollte der mittlere PCS-Durchmesser im Bereich 100 nm bis ungefähr 400 nm, bevorzugt unter 100 nm sein.

Generell ist es günstig, den Wirkstoff zur Herstellung der Dispersion in der Form sehr feiner Kristalle zu verwenden, d.h., in mikronisierter Form mit einer mittleren Teilchengröße im Bereich von ca. 0,1 µm – 25 µm (Kolloidmühle, Gasstrahlmühle). Bevorzugt sind mittlere Teilchengrößen von 0,1µm – 5 µm, besonders bevorzugt von kleiner als 1 µm.

Der pH-Wert der erfindungsgemäßen Dispersionen liegt typischerweise zwischen 4 und 8, vorzugsweise zwischen 5 und 7,5, besonders bevorzugt zwischen 6 und 7,5 und wird in der Praxis bestimmt durch die Applikationsform.

Die Dispersion gemäß der Erfindung kann ferner eine wirksame Menge eines Antioxidanz, wie beispielsweise Vitamin E, insbesondere das Isomer alpha-Tocopherol enthalten. Alternativ

können auch beta- oder gamma-Tocopherol, oder Ascorbylpalmitat verwendet werden. Der Zusatz kann zwischen 10 mg und 2000 mg, vorzugsweise zwischen 25 mg und 1000 mg, bezogen auf 100 g Triglyceride betragen.

Eine typische Dispersion gemäß der Erfindung kann somit, bezogen auf die anwendungsfertige Gesamtzusammensetzung z.B. umfassen: 0,05 bis 1,0 Gew.-%, vorzugsweise 0,05 bis 0,5 Gew.-% des Wirkstoffes, 0,05 bis 2 Gew.-% eines Emulgators oder Emulgatorgemisches, beispielsweise Tween 80 und/oder Ei-Lecithin, dispergiert in einer O/W-Emulsion, die, bezogen auf die Emulsion, 5 bis 30 Gew.-%, vorzugsweise 10 bis 20 Gew.-% Triglyceride enthält. Bei den Triglyceriden handelt es sich vorzugsweise um Sojabohnenöl, mittelkettige Triglyceride (wenigstens 90 % C8/C10) sowie Gemische aus Sojabohnenöl und mittelkettigen Triglyceriden (wenigstens 90 % C8/C10) im Gewichtsverhältnis 1:2 bis 2:1, vorzugsweise 1:1. Daneben können noch, bezogen auf die Gesamtzusammensetzung, 0,5 bis 5 Gew.-%, vorzugsweise 1 bis 3 Gew.-% übliche Isotonisierungsmittel, wie Glycerol, und 0,005 bis 0,05 Gew.-% Antioxidantien, wie beispielsweise alpha-Tocopherol enthalten sein. Ein besonders bevorzugter Wirkstoff ist insbesondere Amphotericin B. Zusätzlich können auch Konservierungsmittel zugesetzt werden. Die trifft insbesondere bei Abpackung der Dispersion in Gefäße zur Mehrfachentnahme zu.

Die Dispersion enthält den Wirkstoff gelöst in einer Menge, die größer ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit jeweils in der Wasser- und der Ölphase der Emulsion ergibt, wobei die "additive Menge" unter Normalbedingungen (20°C, Normaldruck) durch Auflösen der maximalen Wirkstoffmenge in den separaten öligen und wäßrigen Phasen (bei ansonsten identischen Lösebedingungen) entsprechend den Anteilen in der Dispersion ermittelt (Sättigungskonzentrationen) wird.

In der Dispersion sind typische Wirkstoffkonzentrationen 0,01 Gew.-% bis 30 Gew.-%, vorzugsweise 0,1 Gew.-% bis 10 Gew.-%,

besonders bevorzugt 1 Gew.-% bis 5 Gew.-%, bezogen auf die Gesamtmenge der Dispersion.

Arzneistoffe von besonderem Interesse – neben Amphotericin B – sind Vancomycin und Vecuronium. Des weiteren können schwerlösliche Arzneistoffe aus den Gruppen der Prostaglandine, z. B. Prostaglandin E_2, Prostaglandin $F_{2\alpha}$ und Prostaglandin E_1, Proteinase-Hemmstoffe, wie z. B. Indinavir, Nelfinavir, Ritonavir, Saquinavir, Zytostatika, z. B. Paclitaxel, Doxorubicin, Daunorubicin, Epirubicin, Idarubicin, Zorubicin, Mitoxantron, Amsacrin, Vinblastin, Vincristin, Vindesin, Dactiomycin, Bleomycin, Metallocene, z B. Titanmetallocendichlorid, und Lipid-Arzneistoff-Konjugate, wie z. B. Diminazenstearat und Diminazenoleat, und generell schwerlösliche Antiinfektiva wie Griseofulvin, Ketoconazol, Fluconazol, Itraconazol, Clindamycin, insbesondere antiparasitische Arzneistoffe, z. B. Chloroquin, Mefloquin, Primaquin, Pentamidin, Metronidazol, Nimorazol, Tinidazol, Atovaquon, Buparvaquon, Nifurtimox und antiinflammatorische Arzneistoffe, wie z. B.Ciclosporin, Methotrexat, Azathioprin, verwendet werden.

Dispersionen, die antiinflammatorische Arzneistoffe enthalten, können topisch, oral und parenteral angewendet werden. Im Falle einer topischen Anwendung auf der Haut, kann der Arzneistoff in das tiefere Gewebe penetrieren, wo entzündliche Prozesse stattfinden. Mit einer topischen Anwendung auf Schleimhäuten, wie z. B. am Auge, können Erkrankungen wie das "Trockene Auge"-Syndrom behandelt werden, dem ein entzündlicher Prozeß zugrunde liegt. Eine topische Anwendung auf den Schleimhäuten der Vagina ist ebenso vorteilhaft, z. B. ganz besonders für Antiinfektiva. Die Dispersion spreitet gut auf der Schleimhautoberfläche und gewährleistet so eine gleichmäßige Verteilung des Arzneistoffs. Insbesondere wenn diese Dispersion Öltröpfchen und zusätzlich sehr feine Arzneistoffkristalle enthält, da diese feinen Kristalle auf der vaginalen Schleimhaut haften und sich dort langsam auflösen und damit für eine verlängerte Arzneistoff-

wirkung sorgen (Depotwirkung). Für eine Anwendung am Auge ist es vorteilhaft, wenn man Dispersionen verwendet, die positiv geladen sind. Die Wechselwirkungen der positiv geladenen Partikel mit den negativ geladenen Zellmembranen verlängern die Verweilzeit des Arzneistoffs am Wirkort.

Die orale Anwendung der erfundenen Dispersion ist geeignet, die Bioverfügbarkeit von schwerlöslichen Arzneistoffen, die oral nicht ausreichend verfügbar sind, zu erhöhen. Beispiele hierfür sind Paclitaxel und Amphotericin B. Anstelle von wäßrigen Dispersionen können auch, durch Sprühtrocknung oder Gefriertrocknung überführte, trockene Formen verwendet werden.

Die parenterale, insbesondere die intravenöse Anwendung von arzneistoffhaltigen Dispersionen kann Nebenwirkungen reduzieren, z. B. bei Doxorubicin, Daunorubicin und Amphotericin B. Intravenös angewendete Dispersionen können durch Modifizierung der Oberfläche mit Apolipoproteinen gezielt zu gewünschten Zielorganen, wie Gehirn oder Knochenmark gelenkt werden. Dies ist bei Arzneistoffen, die keinen oder nur geringen Zugang zum Gehirn haben, von besonderem Interesse. Typische Beispiele hierfür sind zytotoxische Substanzen wie Doxrubicin. Eine gezielte Aufnahme zytotoxischer Dispersionen in das Gehirn ermöglicht die Behandlung von Hirntumoren, die bisher nur operativ oder lokal, z. B. mit implantierten therapeutischen Systemen und mit arzneistoffhaltigen Implantaten behandelt werden können. Dispersionen, die Antiinfektiva mit geringer Blut-Hirn-Schranken-Permeabilität enthalten, können nun genutzt werden, um diese Antiinfektiva zur Behandlung von persistierenden Parasiten durch die Blut-Hirn-Schranke zu transportieren.

Die Organverteilung von intravenös applizierten Arzneistoffträgern wird von deren physiko - chemischen Eigenschaften, wie z. B. Partikelgröße, Partikelladung und Oberflächenhydrophobie bestimmt. Negativ geladene Partikel werden zum Beispiel wesentlich schneller von den Makrophagen der Leber aufgenommen als

ungeladene Partikel (Wilkens, D, J. and Myers, P. A., Studies on the relationship between the electrophoretic properties of colloids and their blood clearance and organ distribution in the rat. Brit. J. Exp. Path. 47, 568-576, 1966). Um die In-vivo-Organverteilung zu modifizieren, kann die Ladung der erfindungsgemäßen Dispersion geändert werden, speziell positiv geladene Dispersionen sind vorteilhaft. Die positiv geladene Dispersion kann im Bereich der Einstichstelle an den negativ geladenen Zelloberflächen haften bleiben. Nach intravenöser Applikation der negativ geladenen Dispersion interagieren die Partikel mit negativ geladenen Proteinen, speziell mit Albumin, das mengenmäßig bedeutendste Protein im Blut. Aufgrund seiner Funktion als Dysopsonin kann es durch Adsorption an der Tropfenoberfläche und Bildung einer Albumin-Adsorptionsschicht die Verweilzeit der erfundenen Dispersion im Blut verlängern (z. B. verminderte Aufnahme durch Makrophagen der Leber).

Positiv geladene Dispersionen gemäß der Erfindung, können unter Verwendung positiv geladener Emulgatoren, Mischungen von positiv geladenen und ungeladenen Stabilisatoren (z. B. Poloxamere) und/oder negativ geladenen Emulgatoren (z. B. Lecithin) hergestellt werden. Positiv geladene Dispersionen, gemäß der Erfindung, haben ein positives Zetapotential. Das Zetapotential der Dispersionspartikel wird mit elektrophoretischer Messung in destilliertem Wasser (durch Zugabe von Natriumchlorid auf eine Leitfähigkeit von 50 µS/cm eingestellt) oder im Originaldispersionsmedium (äußere Phase der Dispersion) gemessen. Beispiele für positiv geladene Emulgatoren und Stabilisatoren sind Stearylamin, Cetypyridiniumchlorid (CPC), für positiv geladene Lipide N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammoniumchlorid (DOTMA), Didodecyldimethylammoniumbromid (DDAB), 2,3-Dioleyloxy-N-[2(spermidincarboxamid)ethyl]-N,N-dimethyl-1-propylammonium-trifluoroacetat (DOSPA), 3β-[N-(N',N'-Dimethylaminoethan)carbamoyl]-cholesterol (DC-Chol).

Die Herstellung positiv geladener Dispersionen kann unter Verwendung positiv geladener Emulgatoren oder Emulgatormischungen im Produktionsprozeß durchgeführt werden (De novo-Herstellung). Der positiv geladene Emulgator kann alternativ auch zu einer negativ geladenen Dispersion zugefügt werden. Der Emulgator muß in ausreichender Menge zugefügt werden, damit eine Ladungsumkehr von negativ nach positiv eintritt.

Nähere Beschreibung des Produktionsprozesses: Die Mischung aus Lipid, Arzneistoff, Wasser und Emulgator oder andere Stabilisatoren muß einem hochenergetischem Dispergierprozeß unterzogen werden. Sollen Mischungen von Ölen und festen Fetten im Homogenisationsansatz verwendet werden, ist es vorteilhaft, das feste Fett bei erhöhter Temperatur im Öl zu lösen. Die bevorzugte Methode die erfindungsgemäße Dispersion herzustellen, ist die Hochdruckhomogenisation, z. B. mit Kolben-Spalt-Homogenisatoren oder Jet Stream-Homogenisatoren. Befindet sich Wasser in der äußeren Phase der Dispersion, wird die Homogenisation zwischen 0°C und 100°C durchgeführt. Die beste Dispergierung und schnellste Auflösung des schwerlöslichen Arzneistoffs wird erreicht, wenn die Homogenisation deutlich über Raumtemperatur durchgeführt wird, z. B. zwischen 35°C und 100°C. Die optimale Homogenisationstemperatur bei gleichzeitiger Berücksichtigung der chemischen Stabilität des Arzneistoffs wurde zwischen 45°C und 65°C ermittelt. Liegt ein extrem temperaturempfindlicher Arzneistoff vor, sollte die Homogenisation in der Nähe des Gefrierpunktes von Wasser durchgeführt werden (z. B. ungefähr 4°C).

Werden für die äußere Phase der Dispersion andere Flüssigkeiten als Wasser verwendet, die einen höheren Siedepunkt als Wasser besitzen, kann auch bei höheren Temperaturen oder unter 0°C (z. B. PEG 600) homogenisiert werden.

Im Fall von Mischungen aus Lipiden, Mischen von Öl und festem Lipid als "bulk"-Waren kann zu einer festen "bulk"-Mischung

führen – obwohl die daraus in der Dispersion produzierten Partikel flüssig sind (Thomson-Effekt). In diesem Falle sollte die Homogenisation bei einer Temperatur durchgeführt werden, die über dem Schmelzpunkt der "bulk"-Mischung liegt. Daßelbe gilt bei alleiniger Verwendung von festen Lipiden zur Herstellung der Dispersion gemäß der Erfindung. Der angelegte Homogenisationsdruck kann zwischen 10 und 11.000 bar liegen. Werden die Dispersionen mit 11.000 bar produziert, ist die resultierende Dispersion steril, da unter diesem hohen Druck Bakterien und Viren zerrissen werden. Ist eine Sterilisation durch Homogenisation nicht erwünscht, liegt der bevorzugte Produktionsdruck zwischen 200 bar und annähernd 4000 bar. Die in der Industrie in Produktionslinien verwendeten Hochdruckhomogenisatoren arbeiten gewöhnlich in einem Bereich von 200 bar bis 700 bar, daher wäre es nicht notwendig neue Maschinen anzuschaffen, wenn bei diesen Drücken gearbeitet wird. Die Produktion bei niedrigeren Drücken erfordert jedoch eine höhere Anzahl an Durchläufen (Zyklen). Muß eine höhere Anzahl an Durchläufen vermieden werden (z. B. begründet durch Aspekte der chemischen Stabilität des Arzneistoffes), sollte ein höherer Druck angewendet werden, der von 700 bar bis 4000 bar reicht. Für den Bereich 700-1500 bar können Homogenisatoren von APV Gaulin (Lübeck, Deutschland) verwendet werden, für den Bereich 700-2000 bar sind Maschinen der Firma Niro Soavi (Lübeck, Germany) geeignet, des weiteren ermöglichen spezielle Homogenisatoren der Firma Stansted (Stansted, UK) bei Drücken bis zu 4000 bar zu arbeiten.

Um die Dispersion herzustellen kann jede Homogenisatorausstattung verwendet werden, die eine genügend hohe Leistungsdichte erreicht, d. h. typischerweise über 10^4 W/m^3. Bei einigen Homogenisatoren kann die Leistungsdichte (dissipierte Energie pro Volumeneinheit der Dispergierzone) nicht errechnet werden, da die genaue Größe der Dispergierzone nicht bekannt ist (z. B. Microfluidizer). In diesem Fall muß die Eignung der Maschine für die Herstellung der erfundenen Dispersion auf empirischem Wege ermittelt werden. Beispiele für Homogenisatoren vom Kolben-Spalt-

Typ sind die Maschinen von den Firmen APV Gaulin, Niro Soavi, Stansted und French Press, ein Beispiel für Jet Stream-Homogenisatoren ist der Microfluidizer (Microfluidics, Inc., USA).

Die Erfindung wird durch die nachfolgenden Beispiele näher erläutert, ohne sie jedoch zu beschränken.

Beispiele

Beispiel 1

8 mg Amphotericin B wurden mit 40 g Lipofundin N 20 % angerieben (0,2 mg Amphotericin B/ml Emulsion) und die erhaltene Dispersion mit einem Ultra-Turrax-Rührer 5 Minuten bei 8000 Umdrehungen pro Minute gerührt. Anschließend wurde die Dispersion mit einem Micron LAB 40 bei 1.500 bar mit 20 Zyklen hochdruckhomogenisiert. Die Partikelgröße wurde mit einem Laserdiffraktometer bestimmt (Coulter LS 230, Coulter Electronics, USA). Der Durchmesser 50 % (D50%) der Volumenverteilung betrug 0,164 µm, D90% 0,340 µm, D95% 0,387 µm, D99% 0,466 µm und D100% 0,700 µm.

Beispiel 2

Es wurde ein Emulsionssystem mit Amphotericin B wie in Beispiel 1 hergestellt, die eingearbeitete Menge an Amphotericin B betrug jedoch 40 mg (d. h. 1 mg/ml Emulsion). Es wurden folgende Durchmesser gemessen: D50% 0,160 µm, D90% 0,362 µm, D95% 0,406 µm, D99% 0,485 µm und D100% 0,746 µm.

Beispiel 3

Es wurde eine Emulsion analog Beispiel 1 hergestellt, die eingearbeitete Amphotericin B-Menge betrug jedoch 80 mg (d. h. 2 mg/ml Emulsion). Es wurden folgende Durchmesser gemessen: D50%

0,194 µm, D90% 0,381 µm, D95% 0,423 µm, D99% 0,494 µm und D100% 0,721 µm.

Beispiel 4

40 mg Amphotericin B-Pulver wurden mit 40 g Öl (Mischung 50 : 50 aus LCT und MCT) angerieben und die erhaltene Suspension wie in Beispiel 1 mit einem Ultra-Turrax für 5 Minuten gerührt. Anschließend wurde die Suspension mit einem Hochdruckhomogenisator Micron LAB 40 hochdruckhomogenisiert mit 2 Zyklen bei 150 bar, 2 Zyklen bei 500 bar und anschließend 20 Zyklen bei 1.500 bar. 8 g der erhaltenen öligen Suspension wurden dann in 32 g Wasser dispergiert, das 1,2 % Lecithin enthielt. Dispergierung erfolgte mit einem Ultra-Turrax für 5 Minuten bei 8000 Umdrehungen/Minute. Die erhaltene Dispersion wurde dann mit dem Micron LAB 40 hochdruckhomogenisiert bei 500 bar mit 10 Zyklen. Es wurden folgende Durchmesser gemessen: D50% 0,869 µm, D90% 2,151 µm, D95% 2,697 µm, D99% 3,361 µm.

Beispiel 5

Es wurde eine Emulsion analog Beispiel 4 hergestellt, allerdings erfolgte die Herstellung der Emulsion mit Hochdruckhomogenisation nicht bei Raumtemperatur, sondern in einem temperaturkontrollierten LAB 40 bei 50°C. Es wurden folgende Durchmesser gemessen: D50% 0,647 µm, D90% 1,537 µm, D95% 1,768 µm, D99% 2,152 µm und D100% 3,310 µm.

Beispiel 6

Es wurde eine Amphotericin B-Emulsion durch Hochdruckhomogenisation analog Beispiel 1 hergestellt (0,2 mg Amphotericin B/ml Emulsion), die Hochdruckhomogenisation der Emulsion erfolgte bei Raumtemperatur. Der Arzneistoff wurde in 1,2%iger wäßriger Tween 80-Lösung angerieben, die Suspension vorhomogenisiert und 80 mg dieser Suspension mit 40g Lipofundin N 20% gemischt. Es wurden

folgende Durchmesser gemessen: D50% 0,142 µm, D90% 0,282 µm, D95% 0,331 µm, D99% 0,459 µm und D100% 0,843 µm.

Beispiel 7

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Amphotericin B-Konzentration betrug jedoch 1 mg/ml Emulsion. Es wurden folgende Durchmesser gemessen: D50% 0,245 µm, D90% 0,390 µm, D95% 0,426 µm, D99% 0,489 µm, D100% 0,700 µm.

Beispiel 8

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Amphotericin B-Konzentration betrug jedoch 2 mg/ml Emulsion. Es wurden folgende Durchmesser gemessen: D50% 0,237 µm, D90% 0,389 µm, D95% 0,426 µm, D99% 0,491 µm, D100% 0,701 µm.

Beispiel 9

Es wurde eine Emulsion analog Beispiel 6 hergestellt, die Hochdruckhomogenisation der Emulsion erfolgte bei 60°C. Es wurden folgende Durchmesser gemessen: D50% 0,197 µm, D90% 0,388 µm, D95% 0,436 µm, D99% 0,532 µm und D100% 0,953 µm.

Beispiel 10

Es wurde eine Emulsion analog Beispiel 7 hergestellt, der Homogenisationsdruck betrug jedoch 500 bar anstatt 1500 bar. Es wurden folgende Durchmesser gemessen: D50% 0,263 µm, D90% 0,401 µm, D95% 0,435 µm, D99% 0,493 µm und D100% 0,657 µm.

Beispiel 11

Die Partikelgrößenverteilung des Amphotericin B-Pulvers wurde mit Laserdiffraktometrie und Lichtmikroskopie analysiert. Abbildung 1 (oben) zeigt die Teilchengrößenverteilungskurve des Pulvers

nach Dispergierung in Wasser ermittelt mit Laserdiffraktometrie sowie die Partikelgrößenverteilung nach Einarbeitung in das erfindungsgemäße Emulsionssystem aus Beispiel 2 (Abbildung 1, unten). Im Emulsionssystem sind keine Amphotericin B-Kristalle mehr detektierbar, Amphotericin B wurde in das Emulsionssystem inkorporiert.

Beispiel 12

Die Amphotericin B-Emulsion wurde im Vergleich zu in Wasser dispergierten Amphotericin B-Kristallen mit Lichtmikroskopie untersucht. Abbildung 2 zeigt die lichtmikroskopische Aufnahme des Amphotericin B-Pulvers im polarisierten Licht, aufgrund der Anisotropie der Kristalle erscheinen sie hell. Abbildung 3 zeigt die lichtmikroskopische Aufnahme im polarisierten Licht nach Einarbeitung von Amphotericin B in das Emulsionssystem (Beispiel 1), anisotrope Strukturen sind nicht mehr detektierbar, das gesamte Bild ist nahezu schwarz. Für die Lichtmikroskopie wurde das Emulsionssystem unverdünnt auf den Objektträger aufgetragen.

Beispiel 13

Buparvaquon wurde analog zu Amphotericin B wie in Beispiel 6 in ein Emulsionssystem eingearbeitet. Es wurden folgende Durchmesser gemessen: D50% 0,399 µm, D90% 0,527 µm, D95% 0,564 µm, D99% 0,635 µm und D100% 0,843 µm.

Beispiel 14

Atovaquon wurde analog zu Beispiel 1 anstelle von Amphotericin B in ein Emulsionssystem eingearbeitet. Es wurden folgende Durchmesser gemessen: D50% 0,297 µm, D90% 0,437µm, D95% 0,475 µm, D99% 0,540 µm und D100% 0,744 µm.

Beispiel 15

Es wurde eine Emulsion analog Beispiel 1 hergestellt, die Menge an eingearbeitetem Amphotericin betrug jedoch 5 mg/ml Emulsion. Die Löslichkeit in der Dispersion für Amphotericin war überschritten, neben Öltropfen lagen Arzneistoffkristalle vor (heterogene Dispersion).

Beispiel 16

Es wurde eine Amphotericin B-Emulsion durch Zumischung von 40 mg Amphotericin B zu 40 ml Lipofundin N 20 % hergestellt (d. h. Amphotericin B 1 mg/ml Emulsion). Die Mischung wurde mit 10 Zyklen bei 1500 bar und 45°C homogenisiert. Diese Emulsion wurde durch Autoklavieren bei 121°C für 15 Minuten (gemäß Deutschen Arzneibuches) sterilisiert. Der PCS-Durchmesser vor Autoklavierung betrug 203 nm, der Polydispersitätsindex 0,102, nach Autoklavierung lag der Durchmesser bei 208 nm, der Polydispersitätsindex bei 0,137.

Beispiel 17

100 mg Amphotericin B-Pulver wurden in 900 mg sterilen Wasser dispergiert, vorhomogenisiert und unter Verwendung von Pistill und Mörser in 20 g MCT-Öl mit 1,2% Lecithin eingearbeitet. Das Öl wurde in 80 g Wasser dispergiert und diese Mischung in einem Microfluidizer Typ Microfluidix M110y homogenisiert (d. h. Amphotericin B 1 mg/ml Emulsion). Die Homogenisation wurde bei 1000 bar für 10 Minuten durchgeführt. Der PCS-Durchmesser vor Autoklavierung betrug 192 nm, der Polydispersitätsindex 0,113, nach Autoklavierung lag der Durchmesser bei 196 nm, der Polydispersitätsindex bei 0,109.

Beispiel 18

Die unverdünnte Amphotericin B-Emulsion aus Beispiel 17 wurde auf größere Partikel und Amphotericin B-Kristalle mittels Lichtmikroskop untersucht. Abbildung 4 zeigt nur wenige größere Tröpfchen, Amphotericin B-Kristalle konnten nicht detektiert werden.

Beispiel 19

Es wurden Emulsionen, wie in Beispiel 16 beschrieben, hergestellt, wobei jedoch 15 Homogenisationszyklen durchgeführt wurden. Es wurden zwei Dispersionen hergestellt, die 1 mg/ml und 5 mg/ml Amphotericin B enthielten. Die Emulsionen wurden mit Lichtmikroskopie untersucht. Die lichtmikroskopische Aufnahme der Dispersion mit 1 mg/ml zeigt ein Emulsionssystem ohne detektierbare Amphotericin B-Partikel (Abb. 5), in der Dispersion mit 5 mg/ml Amphotericin B sind neben den Emulsionströpfchen kleine Amphotericin B-Kristalle detektierbar (Abb. 6)

Beispiel 20

Es wurde eine Amphotericin B-Emulsion, wie in Beispiel 16, hergestellt. Die Emulsion wurde 20 Zyklen bei einer Produktionstemperatur von 65°C homogenisiert. Der mittlere PCS-Durchmesser betrug 255 nm, der Polydispersitätsindex 0,098. Die Partikelgröße wurde mittels Laserdiffraktometrie mit einem Coulter LS 230 (Coulter Electronics, USA) durchgeführt. Der Durchmesser 50% war 0,247 µm, der Durchmesser 90% 0,410 µm, der Durchmesser 99% 0,566 µm und der Durchmesser 100% 0,938 µm. Die Amphotericin B-Konzentration lag bei 1 mg/ml, Sterilisation wurde mittels Autoklavieren bei 121°C für 15 Minuten durchgeführt. Die Arzneistoffkonzentration wurde mit HPLC analysiert, wobei in zwei Proben 93,8% und 91,0% wiedergefunden wurden.

Beispiel 21

100 mg Cyclosporin wurden mit 40 g Lipofundin N 20% angerieben. Die Homogenisation wurde mit 20 Zyklen bei 1500 bar und 25°C durchgeführt. Der mittlere PCS-Durchmesser betrug 234 nm, der Polydispersitätsindex 0,099. Der Laserdiffraktometerdurchmesser D50% lag bei 0,218 µm, der D90% bei 0,381 µm und der D100% bei 0,721 µm. Mit Lichtmikroskopie konnten keine Cyclosporin-Partikel detektiert werden (polarisiertes Licht, Dunkelfeld). Das Zetapotential der Emulsion wurde in destillierten Wasser mit einer eingestellten Leitfähigkeit von 50 µS/cm (durch Zugabe von Natriumchlorid) gemessen. Die Feldstärke lag bei 20 V/cm, die Umrechnung der elektrophoretischen Mobilität in das Zetapotential erfolgte mit der Helmholtz-Smoluchowski Gleichung. Das Zetapotential betrug -51 mV.

Beispiel 22

Es wurde eine Cyclosporin-Emulsion wie in Beispiel 21 beschrieben hergestellt. Während der Produktion wurden jedoch 0,5% Cetylpyridiniumchlorid (CPC) zugefügt. Die Emulsion war positiv geladen, das Zetapotential betrug +32 mV.

Beispiel 23

Es wurde eine Cyclosporin-Emulsion, wie in Beispiel 21 beschrieben, hergestellt. Während der Produktion wurden jedoch 1,0% Stearylamin zugefügt. Der PCS-Durchmesser betrug 247 nm, der Polydispersitätsindex 0.088. Der Laserdiffraktometerdurchmesser 50% lag bei 0,229 µm, der Durchmesser 90% bei 0,389 µm und der Durchmesser 100% bei 0,721 µm. das Zetapotential betrug +24 mV.

Beispiel 24

Eine Cyclosporin-Emulsion wurde de novo hergestellt. Die Zusammensetzung bestand aus 0,1% Cyclosporin, 0,5% Poloxamer 188, 0,5%

Eilecithin Lipoid E80, 0,15% Stearylamin, 10% Miglyol 812 und 2,25% Glycerol als Isotonisierungszusatz und Wasser ad 100%. Das Lecithin wurde in der Öl-Phase dispergiert, eine Prä-Emulsion wurde unter Zusatz der anderen Bestandteile durch Hochgeschwindigkeitsrühren hergestellt, das Cyclosporin-Pulver wurde im letzten Schritt zugefügt. Diese Mischung wurde bei 45°C mit 20 Zyklen und 1500 bar homogenisiert. Der PCS-Durchmesser betrug 226 nm, der Polydispersitätsindex 0,111. Der Laserdiffraktometer-durchmesser 50% lag bei 0,200 µm, der Durchmesser 90% bei 0,406 µm und der Durchmesser 100% bei 1,154 µm. Die Emulsion war positiv geladen, das Zetapotential betrug +31 mV.

Beispiel 25

Eine O/W-Dispersion wurde produziert mit der Zusammensetzung von 10 g Wasserphase, die 25 mg Amphotericin enthielt, 0,5 g Span 85, 0,25 Tween 80 und Miglyol 812 ad 50 g. 1,0 ml Amphotericin Suspension (2,5% Amphotericin/ml), stabilisiert mit 2,4% Lecithin Lipoid E 80 wurden gemischt mit destilliertem Wasser auf ein Gesamtgewicht von 10 g. Tween 80 wurde zur Wasserphase hinzugefügt, Span 85 zur Ölphase. Das Wasser wurde im Öl durch hochtouriges Rühren dispergiert. Die erhaltene Prä-Emulsion wurde bei 90°C homogenisiert unter Anwendung von 1500 bar und 20 Homogenisationszyklen. Größenanalytik wurde durchgeführt mit Laserdiffraktometrie (Mastersizer E, Malvern Instruments, United Kingdom). Der Durchmesser 50% war 2,25 µm, der Durchmesser 90% 4,21 µm.

Erklärungen zu Abbildungen:

Abb. 1: Partikelgrößenverteilung des Amphotericin-Pulvers vor Einarbeitung in die Dispersion (oben) und Partikelgrößenanalyse der erfindungsgemäßen Dispersion nach Einarbeitung des Amphotericin-Pulvers (unten, Beispiel

2), die Arzneistoffpartikel sind nicht mehr detektierbar (Laserdiffraktometrie)

Abb. 2: Lichtmikroskopische Aufnahme des Amphotericin-Pulvers vor Einarbeitung in die O/W-Emulsion (Beispiel 1) (Polarisations-Aufnahme im Dunkelfeld, anisotrope Kristalle erscheinen weiß, Balken wie in Abb. 3 (10 µm)).

Abb. 3: Lichtmikroskopische Aufnahme der O/W-Emulsion nach Einarbeitung des Amphotericin-Pulvers aus Abb. 2 (Beispiel 1) (Polarisations-Aufnahme, im Dunkelfeld nur schemenhafte Reflexe der isotropen Emulsionstropfen, Balken 10 µm).

Abb. 4: Lichtmikroskopische Aufnahme der unverdünnten Emulsion aus Beispiel 18.

Abb. 5: Lichtmikroskopische Aufnahme der Emulsion mit 1 mg/ml Amphotericin B aus Beispiel 19.

Abb. 6: Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/ml Amphotericin B aus Beispiel 19.

Patentansprüche

1. Dispersion, die eine ölige Phase und eine wäßrige Phase in Form einer O/W-Emulsion oder einer Wasser-in-Öl (W/O) Emulsion, mindestens einen in der öligen und der wäßrigen Phase wenig oder schwer löslichen Wirkstoff sowie gegebenenfalls einen oder mehrere Emulgator(en) und/oder Stabilisator(en) umfaßt, dadurch gekennzeichnet, daß die Dispersion frei von toxikologisch bedenklichen organischen Lösungsmitteln ist und den Wirkstoff gelöst in einer Menge enthält, die höher ist als die Menge, die sich additiv aus seiner maximalen Löslichkeit in der öligen und der wäßrigen Phase der Emulsion ergibt.

2. Dispersion nach Anspruch 1, dadurch gekennzeichnet, daß der Arzneistoff zusätzlich zum gelösten Zustand noch in hochdisperser fester kristalliner Form vorliegt, wodurch sich eine Dispersion mir einer heterogenen dispersen Phase aus Öltropfen und aus Arzneistoffkristallen ergibt.

3. Dispersion nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß mindestens 90%, bevorzugter 95% der vorhandenen Kristalle kleiner als 5 µm sind und insbesondere 100% kleiner als 5µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie), wobei besonders bevorzugt 90% kleiner als 3 µm, bevorzugter 95% kleiner als 3 µm und insbesondere 100% kleiner als 3µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie).

4. Dispersion nach Anspruch 3, dadurch gekennzeichnet, daß mindestens 90%, bevorzugt 95% und insbesondere 99% der Kristalle kleiner als 1 µm sind (Volumenverteilung bestimmt mit Laserdiffraktometrie).

5. Dispersion nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß sie eine Öl-in-Wasser-Emulsion ist und, bezogen auf die Gesamtmenge der Dispersion, 5 bis 99,5 Gew.-%, vorzugsweise 10 bis 95 Gew.-% insbesondere 60 bis 95 Gew.-% und speziell 70-95% wäßrige Phase enthält.

6. Dispersion nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß sie eine Wasser-in-Öl (W/O) Emulsion ist und, bezogen auf die Gesamtmenge der Dispersion, 5 bis 30 Gew.-%, vorzugsweise 10 bis 25 Gew.-% insbesondere 10 bis 20 Gew.-% wäßrige Phase enthält.

7. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie Emulgator und/oder Stabilisator enthält.

8. Dispersion nach Anspruch 7, dadurch gekennzeichnet, daß sie, bezogen auf die Gesamtmenge Dispersion, weniger als 15%, bevorzugt weniger als 10% und insbesondere weniger als 2%, bevorzugt 0,6% bis 1,2% Emulgator und/oder Stabilisator enthält.

9. Dispersion nach einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, daß sie als Emulgatoren Ei-Lecithin, Soja-Lecithin, Phospholipide aus Ei oder Soja, Sorbitanestern (insbesondere Span 85), Polyethylenglykolsorbitanester (insbesondere Tween 80), Natriumglycocholat, Natriumlaurylsulfat (SDS) oder Gemischen derselben und/oder als Stabilisatoren Block-Copolymere, insbesondere Poloxamere (bevorzugt Poloxamer 188 und 407) oder Poloxamine (bevorzugt Poloxamine 908), Polyvinylpyrrolidon (PVP), Polyvinylalkohol (PVA), Gelatine, Polysaccharide (bevorzugt Hyaluronsäure oder Chitosan und ihre Derivate), Polyacrylsäure und ihre Derivate, Polycarbophil, Cellulosederivate (bevorzugt Methyl-, Hydroxypropyl- und Carboxymethylcellulose), Zuckerester (bevorzugt Saccharosemonostearat) oder Natrium-

citrat einzeln oder in irgendeiner Mischung derselben enthält.

10. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie eine O/W-Emulsion umfaßt und die zur Herstellung der Dispersion verwendete ölige Phase (Lipidphase) nur bei Raumtemperatur feste Lipide oder nur bei Raumtemperatur flüssige Lipide umfaßt oder eine Mischung aus einem oder mehreren bei Raumtemperatur flüssigen Lipiden mit einem oder mehreren bei Raumtemperatur festen Lipiden umfaßt.

11. Dispersion nach Anspruch 10, dadurch gekennzeichnet, daß die Mischung aus flüssigem Lipid und festem Lipid von 99 + 1 bis zu 1 + 99 variiert (Gewichtsteile), insbesondere in der Mischung der Anteil von flüssigem Lipid mindestens 10 Teile beträgt, bevorzugt mindestens 30 Teile und insbesondere mindestens 50 Teile.

12. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die Partikel aus folgenden einzelnen Lipiden oder deren Mischungen hergestellt werden: natürliche oder synthetische Triglyceride bzw. Mischungen derselben, Monoglyceride und Diglyceride, alleine oder Mischungen derselben oder mit Triglyceriden, selbst-emulgierende modifizierte Lipide, natürliche und synthetische Wachse, Fettalkohole, einschließlich ihrer Ester und Ether und Mischungen derselben insbesondere synthetische Monoglyceride, Diglyceride und Triglyceride als individuelle Substanzen oder als Mischung, vorzugsweise Hartfett, oder Imwitor 900, Triglyceride, insbesondere Glyceroltrilaurat, Glycerolmyristat, Glycerolpalmitat, Glycerolstearat und Glycerolbehenat, und Wachse, insbesondere Cetylpalmitat, Karnaubawachs und weißes Wachs (DAB), sowie Kohlenwasserstoffe, insbesondere Hartparaffin.

13. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie als Ölphase Sojaöl, Safloröl, langkettige Triglyceride (LCT), mittelkettige Triglyceride (MCT), insbesondere Miglyole, Fischöle und Öle mit einem erhöhten Anteil an ungesättigten Fettsäuren, acetylierte Partialglyceride (bevorzugt wie in Stesolid) einzeln oder in Mischungen enthält.

14. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie als wäßrige Phase Wasser, Mischungen von Wasser mit wassermischbaren organische Flüssigkeiten, insbesondere flüssigen Polyethylenglykolen (PEG) (bevorzugt PEG 400 und 600) enthält.

15. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die wäßrige Phase Zusätze enthält, insbesondere Elektrolyte, Nichtelektrolyte (bevorzugt Glycerol, Glucose, Mannit, Xylit zur Isotonisierung) und/oder Gelbildner (bevorzugt Cellulosederivate zur Viskositätserhöhung).

16. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß die eingesetzte Emulsion eine O/W-Emulsion ist und Lipofundin, Intralipid, Lipovenös, Abbolipid, Deltalipid oder Salvilipid ist.

17. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Arzneiwirkstoffen zur Behandlung des menschlichen und tierischen Körpers.

18. Dispersion nach Anspruch 17, dadurch gekennzeichnet, daß sie einen oder mehrere Arzneistoffe aus den Gruppen der Anaesthetika, Antibiotika, Antimykotika, Antiinfektiva, Kortikoide, Hormone, Antioestrogene, Antispetika, gefäßaktive Substanzen, Glaukomittel, Beta-Blocker, Cholinergi-

ka, Sympathomimetika, Carboanhydrase-Hemmer, Mydriatika, Virustatika, Mittel zur Tumortherapie, Antiallergika, Vitamine, antiinflammatorische Wirkstoffe sowie Immunsuppressiva enthalten, insbesondere Cyclosporin, oder irgendeine Kombination daraus enthält.

19. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie positiv geladen ist.

20. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie positiv geladene Stabilisatoren enthält, insbesondere Natriumlaurylsulfat (SDS), Stearylamin, und/oder positiv geladene Phospholipide und/oder positiv geladene Lipide.

21. Dispersion nach Anspruch 20, dadurch gekennzeichnet, daß sie die eingesetzte Emulsion eine O/W-Emulsion ist und intravenös appliziert werden kann, wobei neben positiven Stabilisatoren auch Mischungen mit Lecithin und/oder nichtionischen Stabilisatoren eingesetzt werden können, insbesondere Poloxamer Polymere.

22. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß sie Cyclosporin als Wirkstoff enthält.

23. Dispersion nach einem der vorhergehenden Ansprüche, dadurch gekennzeichnet, daß als Wirkstoff ein Antimykotikum (bevorzugt Amphotericin B), ein Antiinfektivum (bevorzugt Buparvaquon oder Atovaquon), ein Immunsuppressivum (bevorzugt Cyclosporin A oder eines seiner natürlichen und synthetischen Derivate), ein Mittel zur Tumortherapie (bevorzugt Paclitaxel oder Taxotere) enthält.

24. Verfahren zur Herstellung einer Zusammensetzung gemäß einem der Ansprüche 1 bis 23, dadurch gekennzeichnet, eine wäßrige Phase und eine ölige Phase, die nicht oder nur

teilweise miteinander mischbar sind, sowie gegebenenfalls ein oder mehrere Emulgator(en) und/oder Stabilisator(en) und eine feste Phase, die mindestens einen in der öligen und der wäßrigen Phase wenig oder schwer löslichen Wirkstoff umfaßt, miteinander gemischt werden und die erhaltene Mischung aus flüssigen und festen Phasen einem hochenergetischen Homogenisationsprozeß mit einem Homogenisator unterzogen werden, wobei keine toxikologisch bedenklichen organischen Lösungsmittel verwendet werden.

25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß der Wirkstoff ohne vorherige Auflösung als Feststoff in die flüssigen Phasen der Dispersion eingearbeitet wurde.

26. Verfahren nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß der pulverisierte Wirkstoff mit einer O/W-Emulsion oder einer W/O-Emulsion angerieben oder gemischt wird und diese Prä-Dispersion der Homogenisation oder Hochdruckhomogenisation unterzogen wird.

27. Verfahren nach Anspruch 24 oder 25, dadurch gekennzeichnet, daß der pulverisierte Wirkstoff in einer Emulgatorlösung dispergiert wird, diese Dispersion homogenisiert wird, anschließend mit einer O/W-Emulsion oder einer W/O-Emulsion gemischt wird und die so erhaltene Prä-Dispersion der Homogenisation oder Hochdruckhomogenisation unterzogen wird.

28. Verfahren nach einem der Ansprüche 24 bis 27, dadurch gekennzeichnet, daß als Homogenisator ein Rotor-Stator-Homogenisator (vorzugsweise eine Kolloidmühle) oder ein Hochdruckhomogenisator (vorzugsweise ein Kolben-Spalt-Homogenisator (APV Gaulin, French Press, Niro, Stansted) oder ein Rohrhomogenisator (jet stream) (Microfluidizer oder Nanojet)) eingesetzt wird.

29. Verfahren nach einem der Ansprüche 24 bis 28, dadurch gekennzeichnet, daß der Wirkstoff in einer solchen Menge eingesetzt wird, daß sich der Wirkstoff am Ende des Homogenisationsprozesses vollständig oder nahezu vollständig aufgelöst hat, so daß sich im Lichtmikroskop selbst bei 1000 facher Vergrößerung in 2 von 3 Feldern nicht mehr als 10 Kristalle, vorzugsweise nicht mehr als 5 Kristalle und insbesondere nicht mehr als 1 Kristall nachweisen lassen/läßt.

30. Verfahren nach einem der Ansprüche 24 bis 28, dadurch gekennzeichnet, daß der Wirkstoff in einer solchen Menge eingesetzt wird, daß am Ende des Homogenisationsprozesses neben dem gelösten Anteil des Wirkstoffs noch ein Anteil des Wirkstoffs in ungelöster kristalliner Form vorliegt, der ein Depot bildet.

31. Verfahren nach einem der Ansprüche 24 bis 30, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen Durchmesser 90% kleiner als 5 µm, bevorzugt einen Durchmesser 95% kleiner als 5 µm und insbesondere einen Durchmesser 100% kleiner als 5µm besitzen (Volumenverteilung bestimmt mit Laserdiffraktometrie).

32. Verfahren nach Anspruch 31, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen Durchmesser 90% kleiner als 3 µm, bevorzugt einen Durchmesser 95% kleiner als 3 µm und insbesondere einen Durchmesser 100% kleiner als 3µm besitzen (Volumenverteilung bestimmt mit Laserdiffraktometrie).

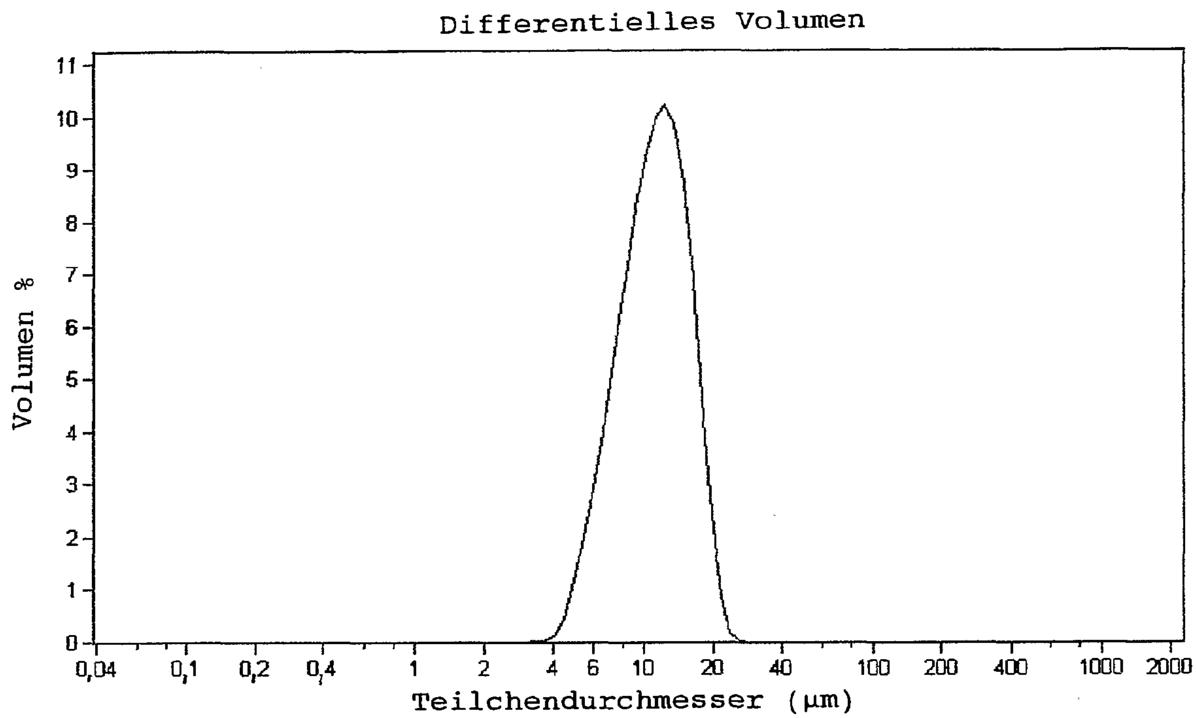
33. Verfahren nach Anspruch 32, dadurch gekennzeichnet, daß die Partikel des Wirkstoffes in ungelöster kristalliner Form einen mit Photonenkorrelationsspektroskopie (PCS) bestimmten Durchmesser kleiner als 1000 nm aufweisen.

34. Verwendung der Dispersion gemäß einem der Ansprüche 1 bis 23 oder hergestellt gemäß einem der Ansprüche 24 bis 33 zur Herstellung eines Arzneimittels.

35. Verwendung nach Anspruch 34, zur Herstellung eines Arzneimittels zur Behandlung von Mykosen, vorzugsweise systemischen Mykosen, Entzündungen, Allergien, Tumorerkrankungen, kardiovaskulären Erkrankungen, viralen und anderen Infektionen und zur Durchführung von Anästhesien.

36. Verwendung nach Anspruch 34 oder 35, dadurch gekennzeichnet, daß das Arzneimittel topisch, oral, peroral sowie parenteral, insbesondere intravenös, intra- und subkutan, intramuskulär, intraartikulär oder intraperitoneal wird, vorzugsweise am Auge angewendet wird und vorzugsweise Cyclosporin enthält.

37. Verwendung nach einem der Ansprüche 34 bis 36, dadurch gekennzeichnet, daß das Arzneimittel eine verlängerte Verweilzeit im Blut zeigt, verglichen mit negativ geladenen Dispersionen.


Differentielles Volumen
Volumen %
11
10
9
8
7
6
5
4
3
2
1
0
0,04
0,1
0,2
0,4
1
2
4
6
10
20
40
100
200
400
1000
2000
Teilchendurchmesser (µm)

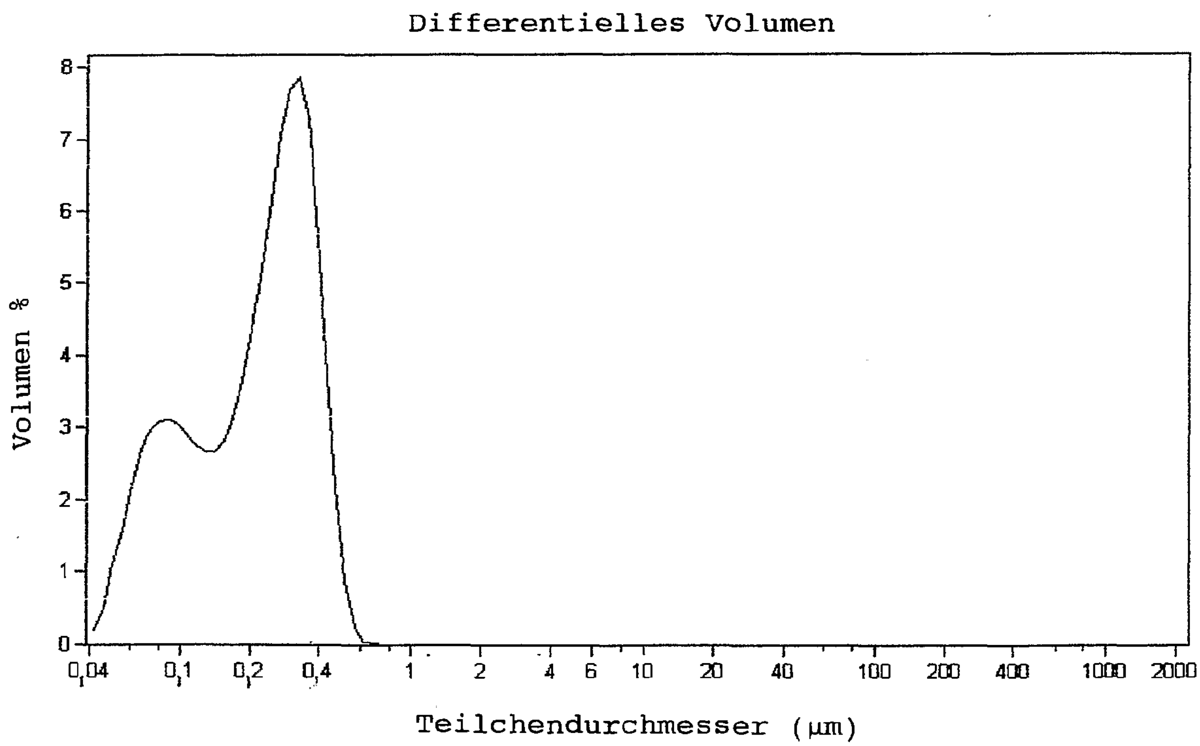

Differentielles Volumen
Volumen %
8
7
6
5
4
3
2
1
0
0,04
0,1
0,2
0,4
1
2
4
6
10
20
40
100
200
400
1000
2000
Teilchendurchmesser (µm)

Abbildung 1

10 µm

Abbildung 2

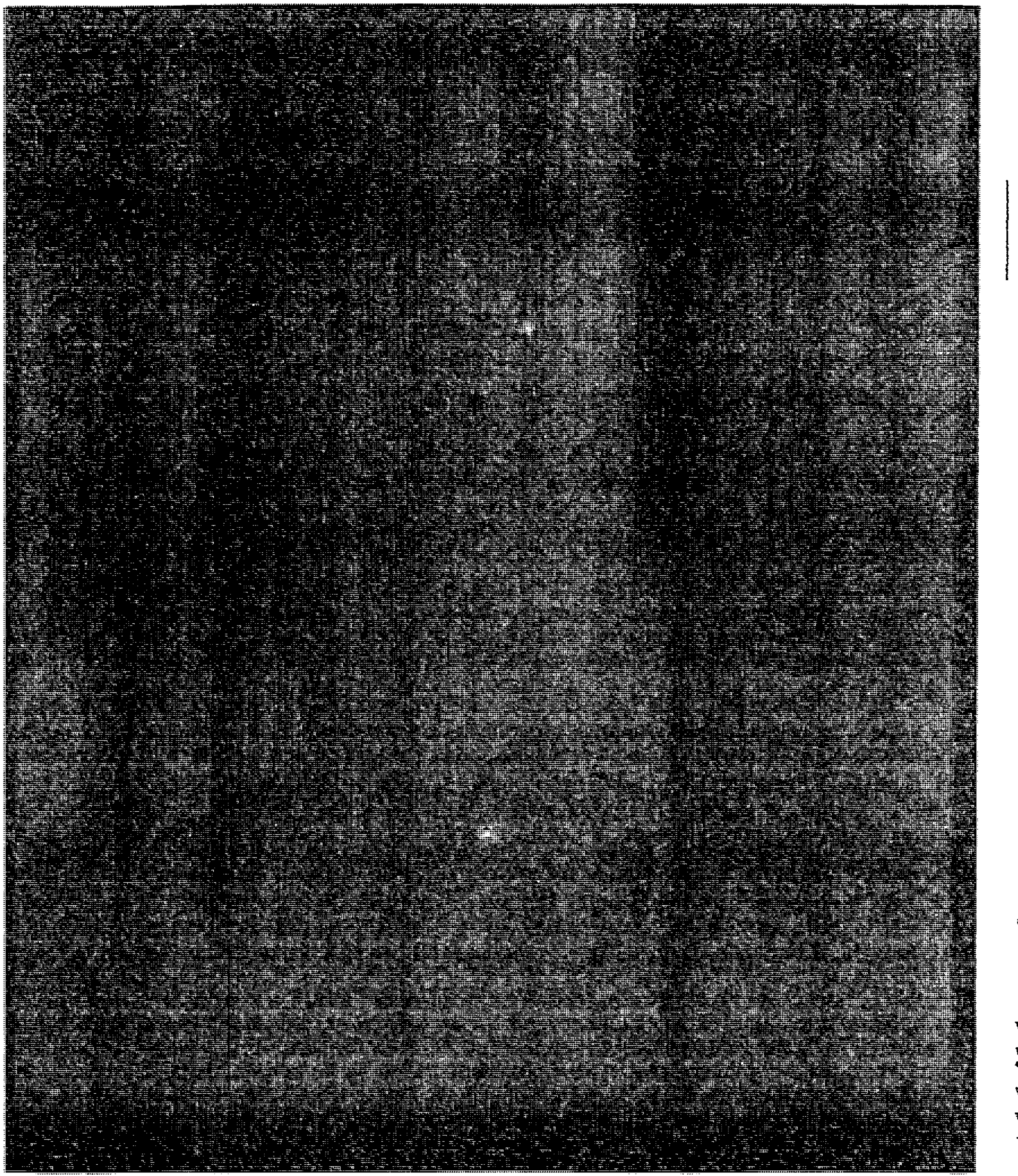


Abbildung 3

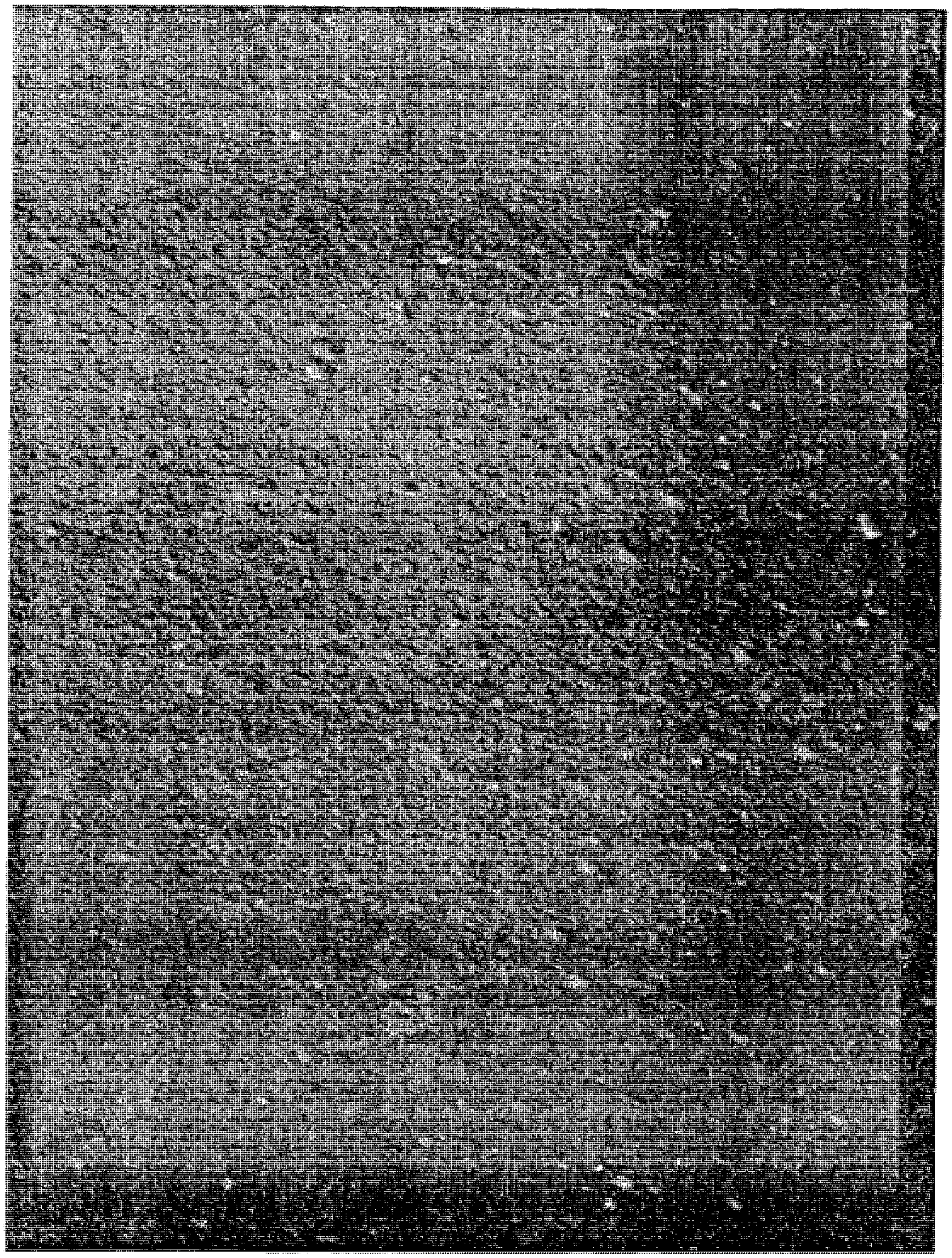


Abb. 4: Lichtmikroskopische Aufnahme der unverdünnten Emulsion aus Beispiel 18.

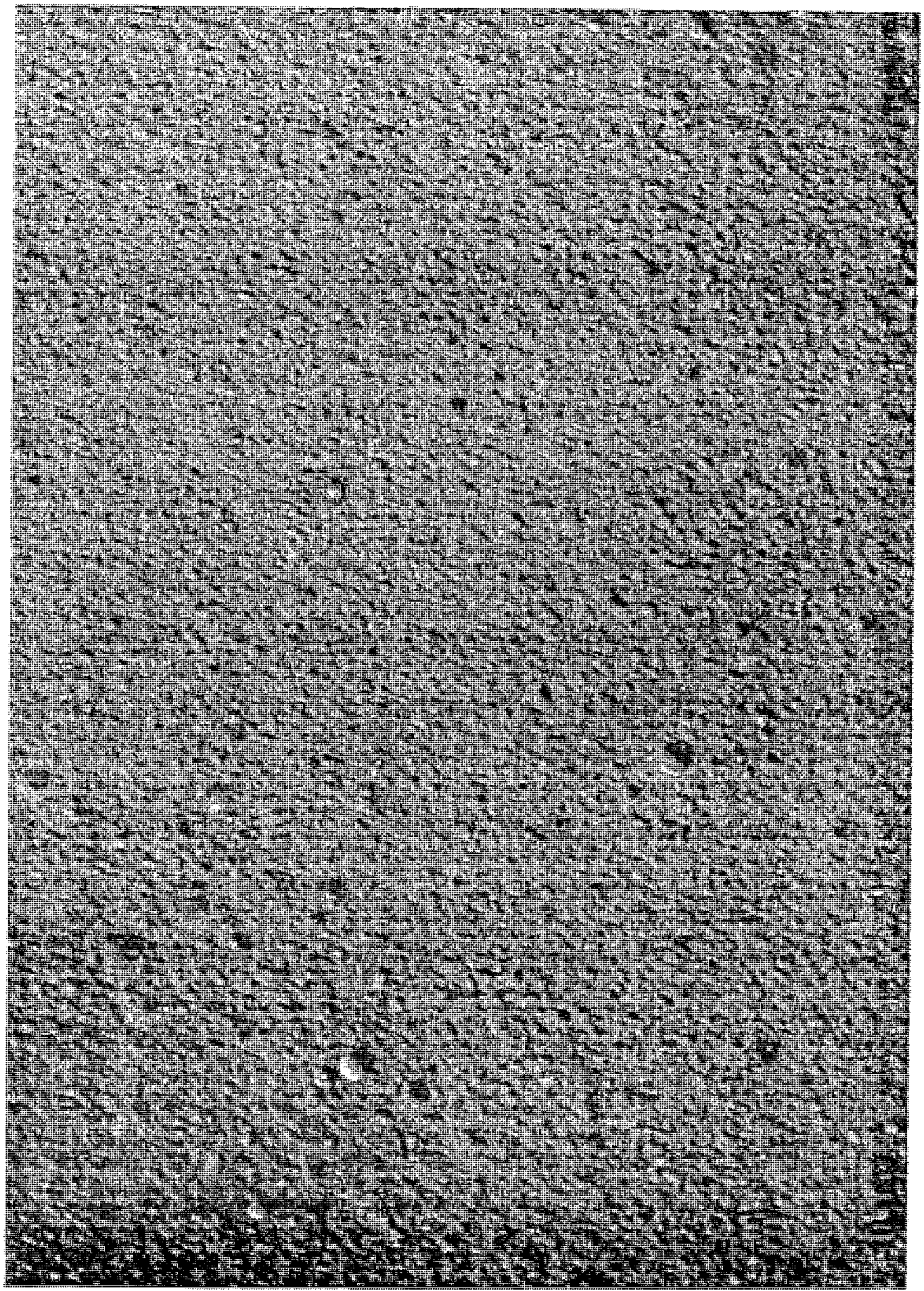


Abb. 5: Lichtmikroskopische Aufnahme der Emulsion mit 1 mg/mL Amphotericin B aus Beispiel 19.

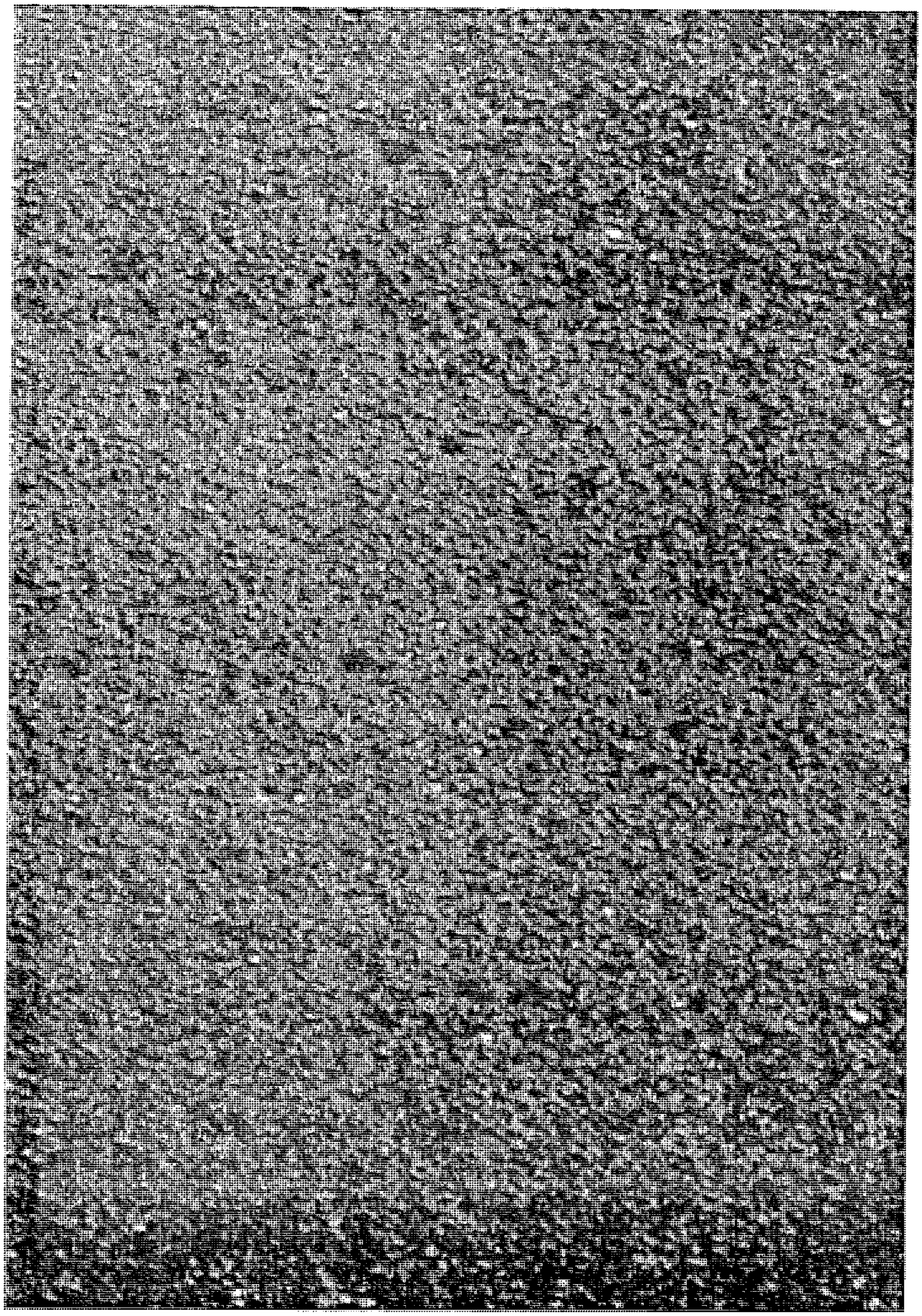


Fig. 6: Lichtmikroskopische Aufnahme der Emulsion mit 5 mg/mL Amphotericin B aus Beispiel 19.

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(54) Title: COMPOSITIONS FOR PREVENTION AND ALLEVIATION OF SKIN WRINKLES

(57) Abstract: The present invention discloses a topical composition for prevention and alleviation of wrinkling which comprises one or two or more selected from the group consisting of Phenytoin, Valproic acid, Cyclosporin A, Nifedipine, Diltiazem, Verapamil HC1 and Amolldipine as an active ingredient having an effect of boosting collagen synthesis.

COMPOSITIONS FOR PREVENTION AND ALLEVIATION OF SKIN WRINKLES

Technical Field

The present invention relates to a topical composition for prevention and alleviation of skin wrinkles which comprises one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis, in conjunction with conventional components of a formulation for transdermal absorption such as cream, ointment, lotion, skin tonic, gel, pack, patch or patch-type administering apparatus.

Background Art

Skin aging is developed by both endogenous causes, for example, aging, and environmental causes. The effects of aging are shown as wrinkles in the skin, which include neck wrinkles, worry lines, frown lines, crow's feet, the folds from the side of the nose to the corners of the mouth, and fine lines around the eyes, below the lips, and over the face. Skin wrinkles caused by aging, though there are individual differences, commonly occur in individuals in their early twenties and increase with age. With aging, the amount of dermal collagen of skin is decreased and alterations in elastic fibers occur, whereby the skin relaxes and fine wrinkles appear. Meanwhile, collagen is a major matrix protein produced by fibroblasts of the skin, being present in the extracellular matrix. It is a primary protein comprising 30 % by weight of proteins in the human body, and has a firm structure of a triple helix. It is known that collagen functions to provide structural stability to the skin, durability of connective tissues and cohesion of tissues while supporting cell coherence, cell proliferation, and induction of differentiation of unspecialized cells. Also, it is known that collagen is broken down by exposure to UV, an

environmental cause of skin aging, and the damage by UV is proportional to the accumulated time of exposure thereto. UV denatures collagenous fibers, causing wrinkles and decreasing elasticity of the skin. Other environmental causes known to promote skin aging include wind, heat and smoking.

As mentioned above, collagen is closely related with skin aging. The amount of collagen in the dermis is decreased with aging and by UV radiation. Collagen decreases by 65 % from age 20 to age 80. Such a decrease of collagen makes the skin thin and further, is closely associated with the formation of skin wrinkles.

Studies have been widely performed to find a method for the prevention and alleviation of skin wrinkles, elucidating important roles of collagen. The studies also elucidated that when collagen synthesis is activated in skin, dermal matrix components are increased, which has effects including alleviation of wrinkles, and increased elasticity and strength of skin. Therefore, using collagen having a moisture retention effect, some collagen-incorporated cosmetics have been developed. Such cosmetics, however, are poor in holding moisture, since the cosmetics are applied to the surface of skin and high molecular weight collagen is poor in transdermal absorption. As a result, their use fails to provide an intrinsic improvement in skin appearance. In the prior art, retinoic acid, TGF-β , protein derived from an animal placenta (JP8-231370), betulinic acid (JP8-208424) and *Chlorella* extract (JP9-40523, JP10-36283) are disclosed as substances for promoting collagen synthesis. As for retinoic acid, it is unstable and has a problem in its safety due to causing irritation and redness upon application the skin, limiting the available dosage thereof. As for other above substances including *Chlorella* extract, their effects of increasing collagen synthesis are weak, so they hardly improve skin appearance. Recently, several new procedures for treating wrinkles by promoting collagen synthesis have been introduced. Examples include ultrasonic treatment, skin scaling, laser peeling, botulinum toxin injection and Restilene injection. These procedures, however, have disadvantages in terms of cost effectiveness and duration of their effects. Thus, it is desirable to search for and develop a

highly effective agent for promoting collagen synthesis.

Disclosure of the Invention

Therefore, the present inventors have conducted studies to develop a compound having an effect of promoting collagen synthesis, and found that phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine which are already known as anticonvulsants, immunosuppressants or calcium channel inhibitors have very strong effects of promoting collagen synthesis in human fibroblast cell lines. Further, it was found that as applied to the skins of rats and mice, the compounds exhibited strong inhibition and alleviation effects of wrinkles, proving the effects of inhibiting and preventing signs of skin aging such as skin wrinkles. Accordingly, the present invention is directed to a composition comprising phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl or amoldipine as an active ingredient having an effect of promoting collagen synthesis.

Phenytoin and valproic acid have been widely used as anticonvulsants for treatment of epilepsy, and their effects on collagen synthesis are documented (USP5686489; Minerva Stomatol., 47(9): 387-398, Sep. 1998). Cyclosporine A has been widely used as an immunosuppressant for suppressing rejection of tissues after transplantation, and its effects on collagen synthesis are reported (J Periodontol., 72(7): 921-931, Jul. 2001). Nifedipine, diltiazem, verapamil HCl and amoldipine have already been used as calcium channel inhibitors, and their effects on collagen synthesis are also reported (J Periodontol., 72(8), Aug. 2001; Proc Natl Acad Sci USA, 93(11): 5478-5482, May 1996; J Urol., 156(6): 2067-2072, Dec. 1996). However, the above drugs are not disclosed for use as topical agents applied to the skin for preventing and alleviating skin wrinkles, as in the present invention.

Hereinafter, a topical composition for preventing and alleviating skin wrinkles will be described in detail, in conjunction with experimental

examples and examples.

Experimental example 1: Effect of active ingredients of the invention on promoting collagen production in fibroblasts

To investigate the effects of active ingredients of the invention on promoting collagen production in fibroblasts in cellular level, respective active ingredients were added to cultures of fibroblasts derived from a human. The synthesized collagen was measured using a modification of a method proposed by Martens (Gut, 33: 1664-1670, 1992) to evaluate the effects of the active ingredients. The experimental protocol in detail is as follows.

Human-derived fibroblasts were transferred to a 24 well plate and cultured in a medium containing 10 % fetal bovine serum (FBS) for 24 hours, followed by washing twice with phosphate buffered saline. The cells were then incubated in a medium containing 1 % FBS in the presence of phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl or amoldipine at final concentrations of 10^{-8} to 10^{-5} M. After 1 hr incubation, cultures were added with 10 μ Ci of ^{3}H-proline per well, followed by a final incubation for 24 hours. After the incubation was terminated, cells from each group were harvested and two fractions of each culture were prepared. One fraction from each culture was treated with collagenase. To all fractions was added trichloroacetic acid to precipitate proteins. The amount of radioactivity incorporated into collagenase-sensitive protein was measured and compared with that of the other fraction which was not treated with collagenase. The difference in radioactivity was attributed to the promoting effect of the compound. Samples without an active ingredient served as a control group, the amount of collagen synthesized being 100 %. The results are shown in Table 1.

Table 1: Effect of promoting collagen production in fibroblasts (%)

Compound /Conc.	Control 1	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	0 M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Phenytoin	100.00	215.28*	298.35	360.65	381.54
Valproic acid	100.00	201.13	283.24	332.11	370.21
Cyclosporine A	100.00	212.11	293.21	352.31	372.27
Nifedipine	100.00	204.31	292.21	330.30	358.16
Diltiazem	100.00	199.15	276.25	321.23	362.12
Verapamil HCl	100.00	183.25	280.23	331.09	355.12
Amoldipine	100.00	182.42	280.07	330.42	355.26

* Rate of collagen production = (collagen production of experimental group/ collagen production of control group) x 100

As shown in Table 1, the active ingredients in experimental groups have effects of promoting collagen production with increasing concentration of the compounds, ranging from the minimum of 182.42 % to the maximum of 381.54 % in a dose-dependent manner, compared to the control group which contains no active ingredient of the invention. This demonstrates that the active ingredients of the invention have excellent effects on promoting collagen synthesis.

<u>Experimental example 2</u>: Promotion of collagen production in rat skin

The effects of application of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on promoting collagen production in animal skin were investigated. The synthesized collagen was measured using a modification of a method proposed by Mard L DaCosta et al. (Surgery, 123: 287-293, 1998).

In brief, 5-week male SD rats were grouped with 5 rats per group. The rats were each incised 1 cm in the center of their abdomens and PVA sponges (Unipoint ind.) were inserted therein. After suturing, as for

experimental groups, respective active ingredients to be examined were applied to the PVA sponge-embeded regions in a volume of 200 μℓ every day for 10 days. Upon autopsy, the PVA sponge was removed to quantify hydroxyproline. The PVA sponge was added with 4 mℓ of 6 N HCl, hydrolized at 130℃ for 3 hours and was subjected to complete drying. 50 μℓ of methanol was added and the solution was incubated at 110℃ until HCl was removed. 1.2 mℓ of 50 % isopropanol was added to dissolve the remaining precipitate. 200 μℓ of chloramine-T (sodium p-toluensulfochloramide trihydrate) solution was added while stirring, and let stand for 10 min. After adding 1.2 mℓ of Ehrlich reagent and mixing, the solution was incubated at 50℃ for 90 min. The resulting solution was cooled to room temperature and absorbance at 558 nm was measured. Hydroxyproline standard solutions were prepared by dissolving 1 mg hydroxyproline in 1 mℓ HCl and diluting it to concentrations of 0, 0.2, 0.4, 0.8, 1 mg each relative to 25 μℓ of 6 N HCl. The standard solutions were hydrolyzed at 130℃ for 3 hours. The quantified value of hydroxyproline, relative to hydroxyproline value (100 %) of the control group which was applied with solvent only, are shown in Table 2.

Table 2: Effect of promoting collagen production in animal skin (%)

Compound /Conc.	Control 1	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	0 M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Phenytoin	100.00	132.58*	143.51	167.41	182.47
Valproic acid	100.00	128.05	139.24	157.72	178.13
Cyclosporine A	100.00	131.02	143.07	164.82	179.26
Nifedipine	100.00	129.92	142.41	161.43	185.88
Diltiazem	100.00	122.44	136.76	157.45	175.23
Verapamil HCl	100.00	135.63	147.39	167.06	183.32
Amoldipine	100.00	132.50	149.65	163.84	181.12

* Rate of collagen production = (hydroxyproline value of experimental group/hydroxyproline value of control group) x 100

As shown in Table 2, the active ingredients increased collagen production in rat skin and the rates of increase ranged from the minimum of 122.44 % to the maximum of 185.88 %, compared to the control group to which no active ingredient of the invention was applied. This demonstrates that the active ingredients of the invention strongly promote dermal collagen synthesis.

Experimental example 3: Effect on inhibiting the generation of wrinkles in hairless mice

The effects of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on inhibiting the generation of wrinkles in hairless mice were investigated.

6-week hairless mice were placed into 21 experimental groups and 3 control groups, with 10 rats per group. For experimental groups, mice were applied to the skin with respective compounds at a concentration of 10^{-8} to 10^{-3} M. The control groups were applied with solvent only, without any active ingredient. The experimental protocol in detail is as follows. Hairless mice were radiated using simulated sunlight at a dose of 2 MED (double Minimal Erythema Dose) 3 days a week for 12 weeks, thereby generating wrinkles. Respective active ingredients or the solvent only were applied twice every day (specifically, on radiation days, the application was performed at 30 min before and after the radiation), at a volume of 100 $\mu\ell$ each for 10 weeks from the first radiation day. Degrees of inhibition of generated wrinkles were determined. The determination was performed by visual observation with naked eyes and photography. The degrees of inhibition of wrinkles in the compound-treatment groups (experimental groups) were compared with the control group (Score 0) and were determined as one of 4 stages, that is, none (Score 0), slight (Score 1), moderate (Score 2) and high (Score 3), and the corresponding mice were counted. The data are shown in Tables 3a to 3c.

Table 3a: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-8} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	2	8
Exp. 2	Valproic acid	0	1	1	8
Exp. 3	Cyclosporine A	0	1	2	7
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	2	2	6
Exp. 6	Verapamil HCl	0	1	1	8
Exp. 7	Amoldipine	0	1	1	8
Control 1	-	10	0	0	0

Table 3b: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-5} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	1	3	6
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	2	2	6
Exp. 6	Verapamil HCl	0	1	2	7
Exp. 7	Amoldipine	0	1	3	6
Control 1	-	10	0	0	0

Table 3c: Effect on inhibiting the generation of wrinkles in hairless mice

Group	Compound (10^{-3} M)	Inhibition of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	0	2	8
Exp. 3	Cyclosporine A	0	0	2	8
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	0	1	9
Exp. 7	Amoldipine	0	0	2	8
Control 1	-	10	0	0	0

As shown in Tables 3a to 3c, the active ingredients inhibited the generation of wrinkles by a high degree in above about 80 % of hairless mice. This demonstrates that active ingredients of the invention have excellent effects on inhibiting the generation of wrinkles.

<u>Experimental example 4</u>: Effect of alleviating wrinkles in hairless mice

The effects of active ingredients of the invention, that is, phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine, on alleviating photo-induced wrinkles in 6-week hairless mice were investigated.

Mice were placed into 21 experimental groups and 3 control groups, with 10 rats per group. For experimental groups, mice were applied to the skin with respective active ingredients at a concentration of 10^{-8} to 10^{-3} M. The control groups were the mice applied with solvent only without any active ingredient. The experimental protocol is as follows. Hairless mice were radiated using a simulated sunlight at a dose of 2 MED (double Minimal Erythema Dose) 3 days a week for 10 weeks, thereby generating wrinkles. Then, respective active ingredients or the solvent only were applied at a volume of 100 $\mu\ell$ each, twice a day for 6 weeks. Degrees of wrinkle

reduction were determined. The determination was performed by visually observing the compound-applied region with naked eyes, and the region was photographed. The degrees of alleviation of wrinkles in the compound-treatment groups (experimental groups) were compared with those of the control group and were determined as one of 4 stages, that is, none (Score 0), slight (Score 1), moderate (Score 2) and high (Score 3), and the corresponding mice were counted. The data are shown in Tables 4a to 4c.

Table 4a: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-8} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	1	2	7
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	2	3	5
Exp. 4	Nifedipine	0	2	3	5
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	2	2	6
Exp. 7	Amoldipine	0	1	1	8
Control 1	-	9	1	0	0

Table 4b: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-5} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	1	3	6
Exp. 2	Valproic acid	0	1	2	7
Exp. 3	Cyclosporine A	0	2	2	6
Exp. 4	Nifedipine	0	2	2	6
Exp. 5	Diltiazem	0	1	2	7
Exp. 6	Verapamil HCl	0	2	1	7
Exp. 7	Amoldipine	0	2	2	6
Control 1	-	9	1	0	0

Table 4c: Effect of alleviating wrinkles in hairless mice

Group	Compound (10^{-3} M)	Reduction of wrinkles (number of mice)			
		Score 0	Score 1	Score 2	Score 3
Exp. 1	Phenytoin	0	0	3	7
Exp. 2	Valproic acid	0	0	2	8
Exp. 3	Cyclosporine A	0	2	2	6
Exp. 4	Nifedipine	0	2	2	6
Exp. 5	Diltiazem	0	0	2	8
Exp. 6	Verapamil HCl	0	1	2	7
Exp. 7	Amoldipine	0	1	3	6
Control 1	-	8	2	0	0

As shown in Tables 4a to 4c, the active ingredients exhibited a high level of alleviation effects on the photo-induced wrinkles in above about 80 % of hairless mice. This demonstrates that active ingredients of the invention have excellent effects on alleviating wrinkles.

The results from the experiments employing the active ingredients of the invention for evaluating effects of promoting collagen synthesis in fibroblasts derived from human, rats and mice demonstrate that phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine with concentrations of 10^{-8} to 10^{-3} M each have excellent effects of promoting collagen synthesis.

A topical composition comprising an active ingredient of the invention may include any formulations applicable to skin, for example, cream, ointment, lotion, skin tonic, gel, pack, aerosol types thereof, patch and patch-type apparatus with micro needles. The compositions were especially prepared in formulations of cream, ointment and pack and applied to human skin for evaluating reduction of wrinkles. It was found that they significantly reduce wrinkle density.

Hereinafter, the present invention will be described in detail, in conjunction with examples and comparative examples. It is noted that these

examples are provided only for illustrative purposes, and the present invention is not to be construed as being limited to those examples.

Preparation of variable formulations comprising an active ingredient of the invention

Agents topically applicable to the skin were prepared with compositions given in Tables 5 to 7, employing each active ingredient and other supplementary components according to the invention. In the invention, ointment, cream, pack, essence, skin softner, nutrient emulsion, patch and patch-type apparatus with micro needles, each topically applicable to the skin, were prepared. It is noted that though only formulations employing phenytoin and cyclosporine A as active ingredients were prepared herein, the examples are not intended to limit the formulations and active ingredients.

Table 5: Formulation of ointment

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Diethyl Sebacate	8	8	8	8	8
Spermaceti	5	5	5	5	5
Polyoxyethylene*	6	6	6	6	6
Sodium benzoate	typical	typical	typical	typical	typical
Phenytoin	0.00001	0.1	-	-	-
Cyclosporine A	-	-	0.00001	0.1	-
Total weight with Vaseline added	100	100	100	100	100

* Polyoxyethylene oleic ether phosphate

Table 6: Formulation of cream

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Stearic acid	15.0	15.0	15.0	15.0	15.0
Setanol	1.0	1.0	1.0	1.0	1.0
Potassium hydroxide	0.7	0.7	0.7	0.7	0.7
Glycerin	5.0	5.0	5.0	5.0	5.0
Propylene glycol	3.0	3.0	3.0	3.0	3.0
Preservative	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Phenytoin	0.00001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 7: Formulation of pack

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Glycerin	5.0	5.0	5.0	5.0	5.0
Propylene glycol	4.0	4.0	4.0	4.0	4.0
Polyvinyl alcohol	15.0	15.0	15.0	15.0	15.0
Ethanol	8.0	8.0	8.0	8.0	8.0
Polyoxyethylene oleic ethyl	1.0	1.0	1.0	1.0	1.0
Paraoxy methyl benzoate	0.2	0.2	0.2	0.2	0.2
Flavor	typical	typical	typical	typical	typical
Phenytoin	0.1	0.5	-	-	-
Cyclosporine A	-	-	0.1	0.5	-
Total weight with purified water added	100	100	100	100	100

Table 8: Formulation of essence

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Cyclometicon	15.0	15.0	15.0	15.0	15.0
Caprilic/capric triglyceride	3.0	3.0	3.0	3.0	3.0
Mineral oil	3.0	3.0	3.0	3.0	3.0
beeswax	1.0	1.0	1.0	1.0	1.0
Cetyl dimethicone copolyol	3.0	3.0	3.0	3.0	3.0
Glycerin	5.0	5.0	5.0	5.0	5.0
Magnesium sulfate	3.0	3.0	3.0	3.0	3.0
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Phenytoin	0.01	0.05	-	-	-
Cyclosporine A	-	-	0.01	0.05	-
Total weight with purified water added	100	100	100	100	100

Table 9: Formulation of skin softner

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Glycerin	2.0	2.0	2.0	2.0	2.0
Hyaluronic acid	1.0	1.0	1.0	1.0	1.0
Polyoxyethylene oleic ether	0.1	0.1	0.1	0.1	0.1
Polyoxyethylene hydrogenated castor oil	0.1	0.1	0.1	0.1	0.1
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Colorant	typical	typical	typical	typical	typical
Phenytoin	0.0001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 10: Formulation of nutrient emulsion

(unit: weight %)

Component	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Setanol	1.0	1.0	1.0	1.0	1.0
Beeswax	0.5	0.5	0.5	0.5	0.5
Vaseline	2.0	2.0	2.0	2.0	2.0
Squalene	6.0	6.0	6.0	6.0	6.0
Ethanol	3.0	3.0	3.0	3.0	3.0
1,3-butyleneglycol	4.0	4.0	4.0	4.0	4.0
Polysolbait 60	1.0	1.0	1.0	1.0	1.0
Solbitan sesqi oleate	0.3	0.3	0.3	0.3	0.3
Carboxy-vinylpolymer	0.3	0.3	0.3	0.3	0.3
Triethanol amine	0.3	0.3	0.3	0.3	0.3
Paraoxy benzoate ester	typical	typical	typical	typical	typical
Flavor	typical	typical	typical	typical	typical
Colorant	typical	typical	typical	typical	typical
Phenytoin	0.0001	0.001	-	-	-
Cyclosporine A	-	-	0.0001	0.001	-
Total weight with purified water added	100	100	100	100	100

Table 11: Formulation of patch

(unit: weight %)

Component	Compound	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Comp.Ex.1
Polymer	carboxymethylcellulose polyacrylic acid	1 2	1 2	1 2	1 2	1 2
Cross link agent	acetaldehyde	0.1	0.1	0.1	0.1	0.1
Humectant	glycerin	30	30	30	30	30
Inorganic filling agent	caolin	0.1	0.1	0.1	0.1	0.1
Preservative	paraoxy methyl benzoate paraoxy propyl benzoate	0.1 0.05	0.1 0.05	0.1 0.05	0.1 0.05	0.1 0.05
Buffer	monosodium phosphate	0.1	0.1	0.1	0.1	0.1
	sodium tripoly phosphate	0.05	0.05	0.05	0.05	0.05
Active ingredient	phenytoin cyclosporine A	0.01 -	0.05 -	- 0.01	- 0.05	- -
Total weight with purified water added		100	100	100	100	100
Support		cotton	cotton	cotton	cotton	cotton
Protective film		silicon	silicon	silicon	silicon	silicon

With regard to a patch-type apparatus with micro needles, a main body of the patch apparatus, a reservoir which contains a solvent for a drug, is comprised of a polymer support for securing an entire patch type apparatus as well as preventing a drug such as phenytoin, valproic acid, cyclosporinee A, nifedipine, diltiazem, verapamil HCl and amoldipine from being permeable thereto. The solvent for a drug may be water, polyethyleneglycol, transcutol or ethanol and is contained inside the reservoir. As for the polymer support, polyethylene, polypropylene, non-woven fabric or cotton fabric are available. The drug mentioned above is dispersed in powdered form in a lower part of the reservoir. The patch-type apparatus is an instrument for administering a drug transdermally, characterized by further comprising a support for micro needles and a number of micro needles. As for a support for micro needles, it is made of a polymer gel such as celluloses, polypropylene, fluorocarbon or polycarbonate and it has a swelling property

as the solvent is released after adhesion to the skin. As for micro needles, they are distributed and fixed perpendicular to the support for micro needles, and they come into contact with the skin. More particularly, 10 to 50 micro needles are attached per unit area (㎠) of the support for needles and each has a channel through which a drug can pass, the channel being 1 to 1000 ㎛ in diameter and the needles being fixed outward 0.01 to 1 ㎜ in length. The apparatus has an adhesive layer at its lower part which has a role in adhering the apparatus to the skin, the adhesive layer being made of a material such as polyacrylate or polybutene. It should be noted that the adhesive layer has no adverse effects on skin and is not dissolved by a solvent. Further, no decrease in adhesive property by a solvent is permitted. Finally, there is a protective film attached to the adhesive layer film, which is easily removable upon using the apparatus, to prevent a drug from being leaked, and protecting an adhesive. Hereinafter, the present invention will be described in detail in conjunction with examples, not to be construed as being limited to those examples.

<u>Preparation of patch-type apparatus with micro needles comprising an active ingredient of the invention</u>

<u>Comparative example 1</u>

1 g of 3 % gelatin solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A comparative matrix was thus obtained.

<u>Example 1</u>

0.001 % phenytoin was added to 1 g of 3 % gelatin solution and homogenously dispersed therein. The solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A phenytoin-dispersed matrix was thus obtained.

Example 2

0.001 % cyclosporine A was added to 1 g of 3 % gelatin solution and homogenously dispersed therein. The solution was poured to a fabric with micro needles (15 needles/㎠) which were fixed perpendicular to the fabric and the resultant fabric was dried under vacuum using a lyophilizer. A cyclosporine A-dispersed matrix was thus obtained.

Evaluation of prevention and treatment effects on skin aging by a composition comprising an active ingredient of the invention

To evaluate the effect of the formulations prepared in above comparative example and examples including the examples as set forth in Tables 5 to 11 on alleviating skin wrinkles, female subjects aged 35 - 60 were employed. 760 females were placed into 38 groups, 20 subjects per group. Respective examples and comparative examples were applied to the face twice per day for 3 months (in case of the packs of Table 7, they were removed 30 min after application). The degrees of alleviating wrinkles were determined by a survey and an image analysis of wrinkles after 3 months. As for the survey, the degrees of alleviation of wrinkles and increase of elasticity were determined as one of 4 stages, that is, none, slight, moderate and high, as compared with the conditions before applying respective compositions, and the corresponding subjects were counted. The data are shown in Tables 12. For the evaluation by an image analysis of wrinkles, one replica of the region right below the eye of each subject was taken using Xantopren (Bayer) before beginning the experiment. Another replica was taken in the same region immediately after finishing the experiment. The replicas were subjected to an image analysis. Wrinkle density was measured by a two dimensional analysis. The measurements were represented as decrease rates, relative to wrinkle densities before the experiment. The results are shown in Table 13.

Table 12: Alleviation of wrinkles in human females

Degree of alleviation	Example	None	Slight	Moderate	High
Ointment	Ex. 1	1*	3	4	12
	Ex. 2	0	2	4	14
	Ex. 3	0	4	7	9
	Ex. 4	0	3	6	11
	Comp. Ex. 1	17	3	0	0
Cream	Ex. 1	0	1	8	11
	Ex. 2	0	1	6	13
	Ex. 3	0	2	7	11
	Ex. 4	0	3	7	10
	Comp. Ex. 1	13	7	0	0
Pack	Ex. 1	0	0	9	11
	Ex. 2	0	2	5	13
	Ex. 3	0	3	4	14
	Ex. 4	0	1	5	14
	Comp. Ex. 1	15	5	0	0
Essence	Ex. 1	0	2	5	13
	Ex. 2	0	0	5	15
	Ex. 3	0	3	6	11
	Ex. 4	0	1	7	12
	Comp. Ex. 1	12	7	1	0
Skin softner	Ex. 1	0	2	8	10
	Ex. 2	0	1	7	12
	Ex. 3	0	3	7	10
	Ex. 4	0	4	5	11
	Comp. Ex. 1	16	4	0	0
Nutrient emulsion	Ex. 1	0	1	5	14
	Ex. 2	0	0	5	15
	Ex. 3	0	1	7	12
	Ex. 4	0	2	7	11
	Comp. Ex. 1	13	7	0	0
Patch	Ex. 1	0	1	4	15
	Ex. 2	0	0	4	16
	Ex. 3	0	1	7	12
	Ex. 4	0	1	9	10
	Comp. Ex. 1	15	5	0	0
Micro-needle patch	Ex. 1	0	1	4	15
	Ex. 2	0	1	9	10
	Comp. Ex. 1	15	5	0	0

*: the number of subjects counted

Table 13: Effect of decreasing wrinkle density in human females

Example	Ointment	Cream	Pack	Essence	Skin softner	Nutrient emulsion	Patch
Ex. 1	45 %	43 %	40 %	39 %	44 %	45 %	46 %
Ex. 2	44 %	41 %	38 %	37 %	42 %	43 %	48 %
Ex. 3	50 %	40 %	41 %	40 %	48 %	46 %	45 %
Ex. 4	48 %	50 %	44 %	39 %	45 %	42 %	44 %
Comp. Ex. 1	98 %	98 %	94 %	97 %	99 %	98 %	96 %

As shown in Table 12, the examples according to the invention provide excellent effects of alleviating wrinkles and increasing skin elasticity. Specifically, more than 80 % showed high levels of improving effects. As shown in Table 13, when the examples comprising an active ingredient of the invention were applied to the subjects, wrinkle densities were considerably decreased to about 37 to 50 %, compared to that before the experiment. Also, when a patch-type administering apparatus with micro needles was applied, examples 1 and 2 exhibited significant decreases in wrinkle densities, 70 % and 60 % respectively, indicating that the examples are superior to the comparative example (98 %) (data not shown).

The above experimental results demonstrate that when the active ingredients of the invention are topically applied to the skin in the form of cream, ointment, lotion, skin tonic, gel, pack, patch, or patch-type apparatus with micro needle, skin wrinkles generated by intrinsic or extrinsic causes are effectively alleviated.

Industrial Applicability

As apparent from the above description, the present invention provides a topical composition which comprises one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis, exhibiting the effects of inhibiting, alleviating and preventing skin aging, such as skin wrinkles.

Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

Claims:

1. A topical composition comprising one or two or more selected from the group consisting of phenytoin, valproic acid, cyclosporine A, nifedipine, diltiazem, verapamil HCl and amoldipine as an active ingredient having an effect of promoting collagen synthesis for prevention and alleviation of skin wrinkles.

2. The composition as set forth in claim 1, wherein the active ingredient is contained at an amount of 0.00001 to 30.00 % by weight, relative to the total weight of the composition.

3. The composition as set forth in claim 1 or claim 2, wherein the composition is formulated in a form of cream, ointment, lotion, skin tonic, gel, pack, patch, or patch-type administering apparatus with micro needles.

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
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Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

CAPLUS(STN), SCISEARCH(STN), PASCAL(STN), BIOTECHNO(STN), INVESTEXT(STN), JICST-EPLUS(STN), KOSMET(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 5686489 A (Tristrata Technology, Inc.) 11 NOV 1997 cited in the application	1-3

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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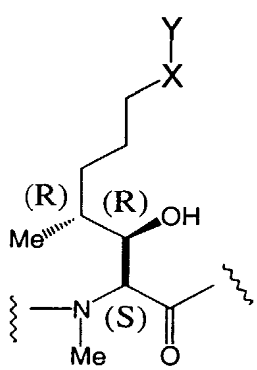


(I)

(57) Abstract: The present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof. In formula (I), the formula for residue A is formula (II), where X is absent, -C1-C6 alkyl-, or -C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH$_2$-OC(O)R2; -C(S)-O-R1; and -C(S)- S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is -(D)Ala-, -(D)Ser- or -[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or -[O-(2-acyloxyethyl)(D)Ser]-. In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or pharmaceutically acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

WO 03/030834 A2

Cyclosporin Analogs for the Treatment of Lung Diseases

Technical Field

The present invention relates to novel cyclosporin analogs and methods for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

Background of the Invention

Respiratory diseases, such as asthma and other diseases characterized by airflow obstruction, are a global problem. Millions of people worldwide, both children and adults, suffer from these medical conditions. These diseases reduce quality of life by impairing the ability of sufferers to perform everyday tasks, and in some cases, cause death. One of the major respiratory diseases is asthma.

Asthma is a disease of unknown etiology in which the bronchi are inflamed and as a consequence obstructed. This narrowing results from a combination of bronchial smooth muscle contraction, mucosal oedema, inflammatory cell infiltrate and partial or total occlusion of the lumen with mucus, cells and cell debris. Bronchial obstruction is either partially or totally reversible, and this important feature distinguishes asthma from chronic bronchitis.

Asthma is an extremely common disease with a worldwide prevalence of 5% to 8%. In the developed world it is the most common chronic illness and, for reasons that are unclear, the disease is on the increase. It is now accepted that asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular, mast cells, eosinophils and T-lymphocytes. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction. This type of airflow obstruction is often reversible either spontaneously or with treatment and causes associated increase in airway responsiveness to a variety of stimuli.

The illness has a wide clinical spectrum ranging from mild episodic bronchospasm (easily controlled by the occasional use of a bronchodilator) to a very severe intractable asthma sometimes resistant to treatment with high doses of

oral corticosteroids. Steroid resistance occurs in less than 5% of people with asthma. This translates to thousands of people. These patients with severe chronic disease may be dependent on corticosteroids and their disease is often so severe that full reversibility can be difficult or impossible to demonstrate.

Chronic obstructive airways disease, chronic obstruction lung disease and 'smoker's chest' have all been used to describe what is now known as COPD. COPD is characterized by progressive irreversible airway obstruction. It can lead to death from respiratory or cardio-respiratory failure. COPD consists of two subsets: chronic bronchitis and emphysema. In practice, it is very difficult to define the contribution of each of these two conditions to the obstruction of the airway and this has led to the displacement of these labels by the non-specific term COPD. The pathology of COPD is not fully elucidated, but features include hypertrophy of mucus-secreting glands, inflammation (including infiltration with lymphocytes) and goblet cell hyperplasia.

The treatment of COPD consists of bronchodilators, intermittent courses of antibiotics and, in some patients, inhaled and/or oral corticosteroids. The latter is claimed to reduce the decline in lung function in COPD.

Cystic fibrosis is an inherited condition. Excess viscid mucus is produced. This leads to recurrent chest infections and progressive bronchiectasis. Approximately 50% of cystic fibrosis sufferers have bronchial hyperresponsiveness and there is an increased incidence of atopy. There is widespread airway narrowing and wheeze. Most cystic fibrosis sufferers take bronchodilators, some take inhaled corticosteroids. And at least one study had reported benefit with oral corticosteroids.

Current drugs for treating asthma are corticosteroids (such as beclomethasone, triamcinolone), beta adrenergics (such as epinephrine, albuterol, bitolterol), NSAIDS, leukotriene antagonists, Xanthines (methyl xanthines such as theophylline, oxtriphylline) and anticholinergics (such as atropine, ipratropium bromide).

Corticosteroids are the mainstay of treatment of chronic asthma and they revolutionized the treatment of this disease when they were first introduced in the 1950's. Oral corticosteroids have today been largely replaced by inhaled corticosteroids, although severe asthmatics still require medication by mouth.

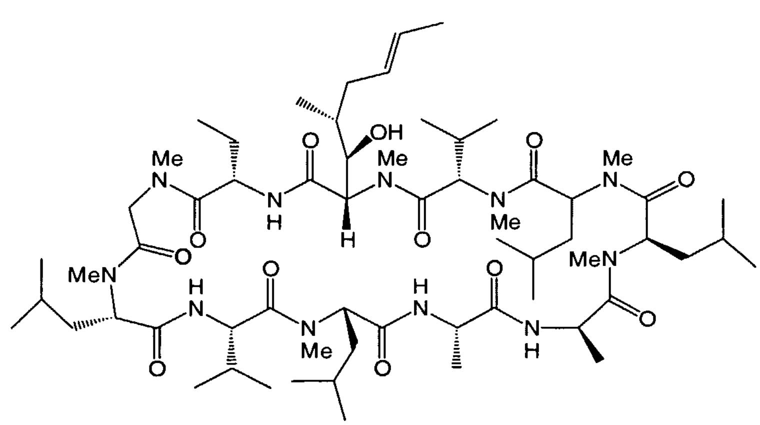
Inhaled cortisteroids are relatively safe and extremely effective in most patients, and improved the quality of life for millions of asthmatic sufferers. For those with severe asthma, however, oral therapy with corticosteroids is required. When taken for more than a few days oral corticosteroids have a number of serious side effects. These include growth retardation in children, severe osteoporosis (especially in old age), decreased responsiveness of the pituitary adrenal axis to stress, fluid retention, diabetes and precipitation of psychosis.

Furthermore, an appreciable number of patients have apparent corticosteroid resistance or unreponsiveness. Patients considered successfully treated with inhaled or oral steroids often have to be content with 60% of their predicted lung function. Further increasing the dose of oral corticosteroids runs the risk of concomitant side effects.

Although corticosteroids are effective for asthma, they are not ideal drugs. Over the years doctors have occasionally used immunosuppressive agents as adjuncts to corticosteroids in patients with extremely severe disease. Examples of immunosuppressive drugs are azathioprine, methotrexate, mycophenolic acid and prodrug, leflunomide, Cyclosporin A, ascomycin, FK-506 and rapamycin.

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological activity, in particular immunosuppressive, anti-inflammatory or anti-parasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite

cyclosporin, *Cyclosporin A* represented as follows:



Since the original discovery of cyclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified, and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprising cyclosporins is thus now substantial and includes, for example, the naturally occurring Cyclosporins A through Z, for example, [Thr]^2, [Val]^2, [Nva]^2 and [Nva]$^{2-}$, [Nva]^5 -Cyclosporin (also known as Cyclosporins C, D, G and M respectively), [(D)MeVal]^{11}-Cyclosporin (also known as Cyclosporin H), [cf., Traber et al.;1, Helv. Chim. Acta, 60, 1247-1255 (1977); Traber et al.; 2, Helv. Chim. Acta, 65, 1655-1667 (1982); Kobel et al.; Europ. J. Applied Microbiology and Biotechnology, 14, 273-240 1982); and Von Wartburg et al.; Progress in Allergy, 38, 28-45, 1986)]; as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporin derivatives and artificial or synthetic cyclosporins including dihydrocyclosporins [in which the MeBmt-residue is saturated by hydrogenation]; derivatized cyclosporins (e.g., in which the 3'-O-atom of the MeBmt- residue is acylated or a further substituent is introduced at the α-carbon atom of the sarcosyl residue at the 3-position); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, for example, [3-O-acetyl-MeBmt]^1–Cyclosporin (also known as Dihydro-cyclosporin D), [(D)Ser]^8-Cyclosporin, [MeIle]^{11}-Cyclosporin, [MeAla]^6-Cyclosporin, [(D) Pro]^3-Cyclosporin etc., employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al., 1; Traber et al., 2; and Kobel et al., loc cit. U.S. Pat. Nos. 4,108,985, 4,220,641, 4,288,431, 4,554,351, 4,396,542 and 4,798,823; European Patent Publication Nos. 34,567A, 56,782A, 300,784A and 300,785; International Patent Publication No. WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, Transpl. Proc., 15 Suppl. 1:2230 (1983); Wenger 2, Angew. Chem. Int. Ed. 24 77 (1985) and Wenger 3, Progress in the Chemistry of Organic Natural Products, 50, 123 (1986).

There is increasing evidence that chronic inflammation in asthma is mediated via a network of cytokines emanating from inflammatory and structural cells in the airways. The prominent eosinophilic inflammation that characterizes asthma appears to be orchestrated by cytokines derived from type 2 T-helper (Th2)-like lymphocytes, suggesting that immunosuppressants might be beneficial in the control of asthma (*see* for example, "Pharmacokinetics, pharmacodynamics, and safety of inhaled cyclosporin A after single and repeated administration in healthy male and female subjects and asthmatic patients," Rohatagi, S. et al., Aventis Pharmaceutical, Collegeville, PA, USA. J. Clin. Pharmacol. (2000), 40(11),

1211-1226). Cyclosporin A (hereinafter "CsA") is active against CD4+ lymphocytes and might, therefore, be useful for asthma. A trial of low-dose oral CsA in patients with steroid-resistant asthma indicated that it can improve control of symptoms in patients with severe asthma on oral steroids.

The mechanism of CsA action in asthma is of interest. CsA binds to the ubiquitous protein cyclophilin, in the cytosol, and the complex in turn binds to calcineurin, which is a calcium and calmodulin dependent serine threonine phosphatase. Calcineurin is necessary for the cytoplasmic portion of the transcription factor NF-AT, a nuclear factor of activated T-cells, to translocate to the nucleus and bind to its nuclear portion to become an active transcription factor. NF-AT forms a complex with AP-1 and regulates the transcription of the IL-2 gene, together with other genes, for example, IL-5. CsA prevents the cytoplasmic portion of NF-AT from translocating, resulting in reduced transcription of IL-2. CsA has a specific inhibitory effect in CD4+ cells through this transcription mechanism, but may also have inhibitory effects on other cells, including mast cells and eosinophils, through mechanisms that have not yet been defined.

Recently, three controlled trials of CsA in asthma have been reported. [Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* **1992**; 339: 324-328; Niwanowska E, Dworski R, Domala B, Pinis G. Cyclosporin for steroid-dependent asthma. *Allergy*, **1991**; 46: 312-315; Lock SH, Kay AB, Barnes NC. Double-blinded, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am J Respir Crit Care Med* **1996**; 153: 509-14; Nizankowska E, Soja J, Pinis G, Bochenek G, Sladek K, Domagala B, et al. Treatment of steroid-dependent bronchial asthma with cyclosporin. *Eur Respir J* **1995**; 8: 1091-1099.]

CsA 5 mg/kg/day allowed a significant reduction in the use of corticosteroids by 60%. Side effects with systemic CsA were increase in diastolic blood pressure and decrease in renal function. Other side effects include hepatic dysfunction, hypertrichosis, tremor, gingival hyperplasis and paraesthesia. The systemic toxicity of CsA limits its use for the treatment of asthma, COPD and other related lung diseases. Therefore, it is desirable to synthesize analogs of CsA which retain CsA's potential utility as a primary or adjunct therapy for respiratory diseases, while reducing or eliminating CsA's systemic toxicity.

Summary of the Invention

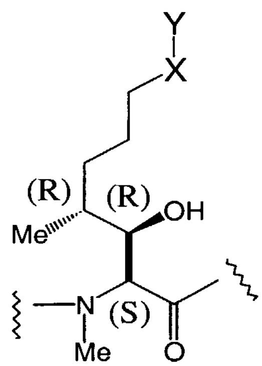
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal
1 2 3 8 11

(I)

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH_2-OC(O)R2; -C(S)-O-R1; and -C(S)-S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or a pharmaceutically

acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject.

In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. In a preferred embodiment, the present invention relates to the processes for the production of cyclosporin analogs of formula I, with the structure of residue A as illustrated above.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

Detailed Description of the Invention

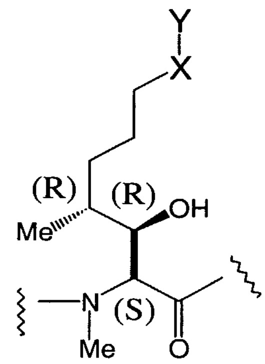
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production. The patents and publications identified in this specification indicate the knowledge in this field and are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure will prevail.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:

$$\boxed{\begin{array}{l} \text{A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal} \\ \;1 \quad\; 2 \quad\; 3 \qquad\qquad\qquad\qquad\qquad\qquad\quad 8 \qquad\qquad\qquad\qquad\quad 11 \end{array}}$$

I

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-OCH$_2$-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; and -C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In formula I, abbreviation of amino acid residues, for example, -Ala-, -MeLeu-, -αAbu-, etc., are in accordance with conventional practice and are to be understood as having the L-configuration unless otherwise indicated (for example, -(D)Ala- represents a residue having the D-configuration). Abbreviation of residues preceded by “Me-” represents a α-N-methylated amino acid residue, for example, “Me-Leu” is a α-N-methylated-Leucine residue. Individual residues of a molecule of the cyclosporin analog of the present invention are numbered, as in the art, clockwise and starting with the residue -MeBmt-, corresponding to residue 1. The same numerical sequence is employed throughout the present specification and claims.

In a most preferred embodiment, a cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where residue B is -αAbu- and residue U is -(D)Ala-. In another preferred embodiment, the cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where X is absent in residue A, residue B is -αAbu- and residue U is - (D)Ala-.

Representative compounds of the invention include, but are not limited to, the following compounds as illustrated below:

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOH; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2Cl$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2OCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-COOCH_2OCH_2CH_2O\ CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = $-C(=O)SCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is $-CH_2CH_2CH_2-$ and Y = $-COOCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOFmoc; residue B = –αAbu-, and residue U = –(D)Ala-.

Cyclosporin analogs of the invention are accordingly useful for the treatment of diseases or conditions responsive to or requiring topical anti-inflammatory, immunosuppressive or related therapy, for example, topical administration for the treatment of such diseases or conditions of the eye, nasal passages, buccal cavity, skin, colon or, especially, airways or lung. In particular, cyclosporin analogs of the invention permit topical anti-inflammatory, immunosuppressive or related therapy with the concomitant avoidance or reduction of undesirable systemic side effects, for example general systemic immunosuppression.

Cyclosporin analogs of the invention useful for the treatment of diseases and conditions of the airways or lung, in particular, inflammatory or obstructive airway diseases. They are especially useful for the treatment of diseases or conditions of the airways or lungs associated with or characterized by inflammatory cell infiltration or other inflammatory events accompanied by inflammatory cell accumulation, for e.g., eosinophil and/or neutrophil. Most preferably, they are useful for the treatment of asthma.

Cyclosporin analogs of the invention are useful in the treatment of asthma of whatever type of genesis including both intrinsic and, especially, extrinsic asthma. They are useful for the treatment of atopic and non-atopic asthma, including allergic asthma, bronchitic asthma, exercise induced asthma, occupational asthma, asthma induced following bacterial infection and other non-allergic asthmas. Treatment of asthma is also to be understood as embracing treatment of "wheezy-infant syndrome," that is treatment of subjects, for example, of less than 4 to 5 years of age, exhibiting wheezing symptoms, in particular at night, and diagnosed or diagnosable as "wheezy infants," an established patient category of major medical concern and now more correctly indentified as incipient or early-phase asthmatics. Cyclosporin analogs of the invention are in particular useful for the treatment of asthma in subjects whose asthmatic status is either steroid dependent or steroid resistant.

Cyclosporin analogs of the invention are also useful for the treatment of bronchitis or for the treatment of chronic or acute airways obstruction associated therewith. Cyclosporin analogs of the invention may be used for the treatment of bronchitis of whatever type or genesis, including, for example, acute bronchitis,

arachidic bronchitis, catarrhal bronchitis, chronic bronchitis, croupous bronchitis, phthinoid bronchitis and so forth.

Cyclosporin analogs of the invention are in addition useful for the treatment of pneumoconiosis (an inflammatory, commonly occupational, disease of the lungs, frequently accompanied by airways obstruction, whether chronic or acute, and occasioned by repeated inhalation of dusts) of whatever type or genesis, including, for example, aluminosis, anthracosis, asbestosis, berylliosis, chalicosis, ptilosis, siderosis, silicosis, tabacosis and, in particular, byssinosis.

Cyclosporin analogs of the invention may also be used for the treatment of eosinophil-related disorders of the airways (e.g. involving morbid eosinophilic infiltration of pulmonary tissues) including hypereosinophilia as it effects the airways and/or lungs as well as, for example, eosinophil-related disorders of the airways consequential or concomitant to Loffler's syndrome, eosinophilic pneumonia, parasitic (in particular metazoan) infestation (including tropical eosinophilia), bronchopulmonary aspergillosis, polyarteritis nodosa (including Churg-Strauss syndrome), eosinophilic granuloma and eosinophil-related disorders affecting the airways occasioned by drug-reaction.

The word "treatment" as used herein in relation to the treatment of diseases of the airways and lungs, in particular asthma, is to be understood as embracing both symptomatic and prophylactic modes, that is for immediate treatment, for e.g., of acute inflammation (symptomatic treatment) as well as for advance treatment to prevent, ameliorate or restrict long term symptomatology (prophylactic treatment). The term "treatment" as used in the present specification and claims in relation to such diseases is to be interpreted accordingly as including both symptomatic and prophylactic treatment, for e.g., in the case of asthma, symptomatic treatment to ameliorate acute inflammatory events and prophylactic treatment to restrict on-going inflammatory status and to ameliorate future bronchial exacerbation associated therewith.

Cyclosporin analogs of the invention may also be used to treat any disease or condition of the airways or lungs requiring immunosuppressive therapy, for e.g., the treatment of autoimmune diseases, or as they affect, the lungs (for example, for the treatment of sarcoidosis, alveolitis or chronic hypersensitivity pneumonitis) or for the maintainance of allogenic lung transplant, for e.g., following lung or heart lung transplantation.

As previously indicated, for the above purposes, cyclosporin analogs of the invention will be administered topically within the airways, for e.g., by the pulmonary route or by inhalation. As also previously noted, while having potent efficacy when administered topically, cyclosporin analogs of the invention exhibit reduced systemic toxicity. Cyclosporin analogs of the invention thus provide a means for the treatment of diseases and conditions of the airways or lung, for example, as hereinabove set forth, with the avoidance of unwanted systemic side effect, e.g. consequent to inadvertent swallowing of drug substance during inhalation therapy. It is estimated that during the course of manoeuvres required to effect administration by inhalation, up to 90% or more of total drug substance administered will normally be swallowed rather than inhaled.

By the provision of cyclosporin analogs which are topically active, e.g. effective when inhaled, but systemically inactive the present invention makes cyclosporin therapy available to subjects for whom such therapy might otherwise be excluded, e.g. due to the risk of systemic, in particular immunosuppressive, side effect.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' opthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, multiple myeloma, etc.; obstructive airway diseases, which includes conditions such as COPD asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis, allergic rhinitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly

following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

The compounds of the present invention may also find utility in the chemosensitization of drug resistant target cells. Cyclosporin A and FK-506 are known to be effective modulators of P-glycoprotein, a substance which binds to and inhibits the action of anticancer drugs; by inhibiting P-glycoprotein, they are capable of increasing the sensitivity of multidrug resistant (MDR) cells to chemotherapeutic agents. It is believed that the compounds of the invention may likewise be effective at overcoming resistance expressed to clinically useful antitumour drugs such as 5-fluorouracil, cisplatin, methotrexate, vincristine, vinblastine and adriamycin, colchicine and vincristine.

Accordingly, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a cyclosporin analog of the invention in combination with a pharmaceutically acceptable carrier or excipient. In particular, compositions pertaining to the present invention are useful for treating a subject for a reversible obstructive airway disease.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients, as described throughout the present specification. Such treatment of the disease may be done by administering a therapeutically effective amount of a compound of the invention for such time and in such amounts as is necessary to produce the desired result.

As used in the present invention, "therapeutically effective amount" of one of the compounds means a sufficient amount of the compound to treat a particular disease, at a reasonable benefit/ risk ratio. The compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug forms. Alternatively, the compound may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more drugs or pharmaceutically acceptable excipients. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Dosages of the cyclosporin analogs of the present invention employed in practicing the method of the present invention will of course vary depending on the site of treatment, the particular condition to be treated, the severity of the condition, the subject to be treated (for e.g., in terms of body weight, age and so forth) as well as the effect desired. In general, for treating diseases or conditions of the airways or lungs, for e.g., inflammatory or obstructive airway disease such as asthma, cyclosporins of the invention can be suitably administered topically to the airways or lungs, for e.g., but not limited to, inhalation, at dosages from about 20 to about 400 mg/day, preferably from about 50 to about 300 mg/day, most preferably from about 200 to about 300 mg/day. Dosages will appropriately be administered from a metered delivery system in a series of from 1 to 5 puffs at each administration, with administration performed once to four times daily. Dosages at each administration will thus conveniently be from about 5 to 100 mg/day, more preferably from about 12.5 to about 100 mg/day, e.g. administered with a metered delivery device capable of delivering, for e.g., 1 to 25 mg cyclosporin per actuation. For purposes of oral administration, more preferable doses may be in the range from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

<u>Definitions</u>

The terms "C_1-C_3-alkyl" or "C_1-C_6-alkyl" as used herein refer to saturated, straight- or branched-chain hydrocarbon radicals containing between one and

three or one and six carbon atoms, respectively. Examples of C_1-C_3 alkyl radicals include methyl, ethyl, propyl and isopropyl, and examples of C_1-C_6-alkyl radicals include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl and n-hexyl.

The term "C_1-C_6-alkoxy" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom. Examples of C_1-C_6-alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, *tert*-butoxy, neopentoxy and n-hexoxy.

The term "C_1-C_6-alkylthio" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through a sulfur atom. Examples of C_1-C_6-alkylthio include, but are not limited to, thiomethoxy, thioethoxy, thiopropoxy, thio-isopropoxy, *n*-thiobutoxy, *tert*-thiobutoxy, neothiopentoxy and n-thio-hexoxy.

The term "aryl" as used herein refers to a carbocyclic ring system having one or more aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups (including multi-cyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from lower alkyl, substituted loweralkyl, haloalkyl, alkoxy, thioalkoxy, lower alkylenedioxy, lower alkylidenedioxy, amino, alkylamino, dialkylamino, acyamino, cyano, hydroxy, acyl, halo and/or trifluoromethyl, mercapto, nitro, carboxylaldehyde, carboxy, alkoxycarbonyl, carbamoyl, sulfamoyl, lower alkoxycarbonylamino, lower alkanoyl, ureido, amidino and carboxamide. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "C_3-C_6-cycloalkyl-" as used herein refers to carbocyclic groups of 3 to 6 carbons, respectively; for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

The term "heterocyclics", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined

to the rest of the molecule via any of the ring atoms, such as, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

The term “subject” as used herein refers to a mammal or animal. Preferably the mammal is a human. A subject refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs and the like.

The term “pro-drug” as used herein refers to pharmacologically acceptable derivatives, for example, but not limited to, esters and amides, such that the resulting biotransformation product of the derivative is the active drug. Pro-drugs are known in the art and are described generally in, e.g., Goodman and Gilman’s “Biotransformation of Drugs,” in the Pharmacological Basis of Therapeutics, 8th Ed., McGraw Hill, Int. Ed. 1992, page 13-15, which is hereby incorporated by reference in its entirety.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate,

oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Pharmaceutical Compositions

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a compound of the present invention formulated together with one or more pharmaceutically acceptable carriers. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminun hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgement of the formulator. The pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or as an oral or nasal spray.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert

diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required.

Pharmaceutically acceptable diluents or carriers may be diluents or carriers acceptable for topical application at the intended site of therapy, e.g. diluents or carriers acceptable for topical administration pulmonary, dermally, nasally, ocularly or rectaly.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide,

calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

Forms in topically administrable form, e.g. enabling or facilitating topical administration, include, e.g. dry powder preparations of the active ingredient (i.e. cyclosporin analog of the invention) in substantially pure form, for example as employed in the art for delivery from a dry powder inhalation device. Means or devices enabling or facilitating topical administration include, in particular, inhalation devices as well as containers and the like from which the active ingredient may be delivered in a form capable of topical application. Preferred embodiments as defined under C will be such as permit topical administration within the airways or lungs, e.g. by inhalation.

It is clear that safety may be maximized by delivering the drugs by the inhaled route either in nebuliser form or as dry powder. Clearly the great advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or of chronic sinusitis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

Preparation of forms suitable for administration by inhalation may be carried out by methods known in the art. It should be noted that several antibiotics have recently developed for topical inhaled usage, particularly in cystic fibrosis, where they have been shown to be effective against pseudomonas infections. Various inhalants are described. For example, in DE 1491707, GB 1,392,945, GB 1,457,351, GB 1,457,352, NL 147939, DE 1491715, GB 1,598,053, EP 5585, EP 41783, EP 45419, EP 360463 and FR 2628638. DE 1491715, in particular, is said to be suitable for inhalation therapy intended for bronchial or lung diseases.

For this purpose cyclosporin analogs of the invention may be employed in any suitable finely dispersed or finely dispersible form, capable of administration

into the airways or lungs, for example in finely divided dry particulate form or in dispersion or solution in any appropriate (i.e. pulmonarily administerable) solid or liquid carrier medium. For administration in dry particulate form, cyclosporin analogs of the invention may, for example, be employed as such, i.e. in micronised form without any additive materials, in dilution with other appropriate finely divided inert solid carrier or diluent (e.g. glucose, lactose, mannitol, sorbitol, ribose, mannose or xylose), in coated particulate form or in any other appropriate form as know in the art for the pulmonary administration of finely divided solids.

Pulmonary administration may be effected using any appropriate system as known in the art for delivering drug substance in dry or liquid form by inhalation, e.g. an atomizer, nebulizer, dry-powder inhaler or like device. Preferably a metered delivery device, i.e. capable of delivering a pre-determined amount of cyclosporin analog at each actuation, will be employed. Such devices are known in the art.

For nasal administration, cyclosporin analogs of the invention will suitably be administered in liquid form from a nasal applicator. Suitable topical forms for the treatment of diseases or conditions of the skin will include, for example, creams, gles, ointments, pastes, cataplasms, plasters, transdermal patches and the like. Formulations for dermal application will appropriately contain a skin penetration enhancer, e.g. as know in the art, for example azone. Forms suitable for ophthalmic use will include lotions, tinctures, gels, ointment and ophthalmic inserts, again as known in the art. For rectal administration, i.e. for topical therapy of the colon, cyclosporin analogs of the invention may be administered in suppository or enema form, in particular in solution, e.g. in vegetable oil or like oily system for use as a retention enema.

According to the present invention, cyclosporin analogs may be used for the manufacture of a topical preparation for the treatment, with or without the concurrent use of other drugs. For the above purposes, cyclosporin analogs of the invention may be employed in any dosage form appropriate for topical administration to the desired site. For example, for the treatment of diseases of the airways or lungs, cyclosporin analogs of the invention may be administered via the pulmonary route, by inhalation from an appropriate dispenser device.

Dosage for the topical preparation will in general be one tenth to one hundredth, of the dose required for oral preparation.

Abbreviations

Sar:	Sarcosine
MeLeu:	N-Methyl-Leucine
Val:	Valine
Ala:	Alanine
MeVal:	N-Methyl Valine
Et:	Ethyl
Ph:	Phenyl
Fmoc:	9-Fluorenylmethoxycarbonyl-
MeBmt:	N-Methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine
α-Abu:	α-Aminobutyric acid

Synthetic Methods

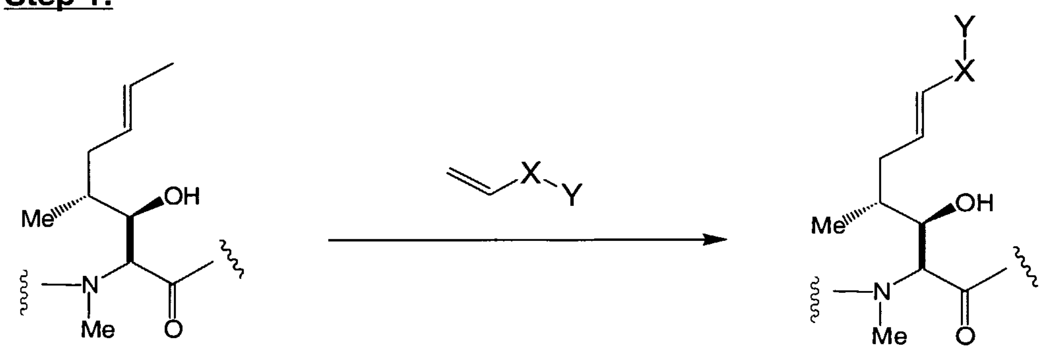
The compounds and processes of the present invention will be better understood, but are not limited to, the following synthetic scheme which illustrates the methods by which the compounds of the present invention (illustrated by formula I) may be prepared. The groups X and Y, and the amino acid residues B and U in formula I are as defined earlier in the specification. The starting material for Scheme I, illustrated by formula I where A'= -MeBmt-, may be, for example, but not limited to, a fermentation product or a synthetic product made by solution phase chemistry. Preferably, the starting material is commercially available. The starting material as a fermentation product may be made from highly productive strains, for example, but not limited to, *Sesquicillopsis rosariensis* G. ARNOLD F605; *Tolypocladium inflatum* wb6-5; Fusant, *Tolypocladium inflatum* KD461 etc. (in U.S. Patent Nos. 5,256,547; 5,856,141 etc.). Alternately, the starting material may be made by solution phase chemistry either by sequentially assembling amino acids or by linking suitable small peptide fragments, where the units are linked by, for example, but not limited to, amide, ester or hydroxylamine linkages (described in, *Müller, Methoden der organischen*, Chemie Vol. XV/2, pp 1 to 364, Thieme Verlag, Stuttgart, 1974; Stewart, Young, Solid Phase Peptide Synthesis, pp 31 to 34, 71 to 82, Pierce Chemical Company, Rockford, 1984; Bodanszky, Klausner, Ondetti, Peptide Synthesis, pp 85 to 128, John Wiley & Sons, New York, 1976 and other standard books on solution phase peptide chemistry). For amide linkages particular preference is given to the azide method, the symmetric and mixed anhydride method, *in situ* generated or preformed active esters and methods using

coupling reagents (e.g., dicyclohexylcarbodiimide, N,N-dimethyl-4-aminopyridine, N-hydroxy-benzotriazole, PyBrop® etc.). Classical solution phase chemistry using standard Z- and Boc- methodology may be used.

Residue A, which is -MeBmt- in the starting material is further modified, as illustrated in the following reaction scheme.

Scheme:

Step 1:



A' = -MeBmt-

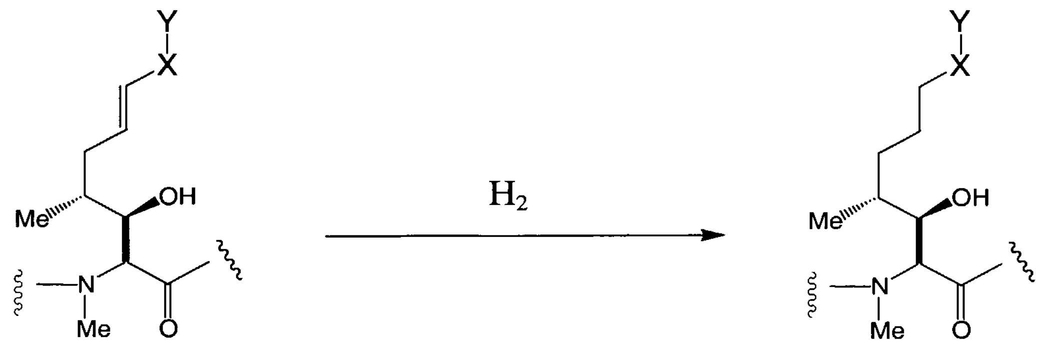
A", wherein X, Y are as defined

(i)

The process for the preparation of the compounds of formula I comprises reacting a compound of formula I, where A' = -MeBmt- (for example, Cyclosporin A) with an olefin having a terminal double bond with catalysts such as Grubb's ruthenium alkylidene, Grubbs dihydroimidazole ruthenium, Shrock-Hoveyda molybdenum catalysts or benzylidene catalysts [*see* (a) US Patent 6,111,121; (b) Reviews: Synlett, **1999**, *2*, 267; (c) Reviews: Ivin, K J; Mol, J.C. *Olefin Metathesis and Metathesis Polymerization*, 2nd ed., Academic Press, New York, **1997**; (d) *J. Org. Chem.*, **1999**, *64*, 4798-4816; (e) *Angew. Chem.*, Int. Ed. English, **1997**, *36*, 2036-2056; (f) *Tetrahedron* **1998**, *54*, 4413-4450.] or Nolan's ruthenium catalyst [see (a) International Patent Application No. WO 00/15339; (b) *Org. Lett.*, **2000**, *2*, 1517-1519; (c) *J. Org. Chem.*, **2000**, *65*, 2204-2207] or Molybdenum catalysts [see (a) *J. Am. Chem. Soc.*, **1990**, *112*, 3875 (b), *J. Am. Chem. Soc.*, **1996**, *118*, 10926-10927] in the presence of a lithium salt such as lithium bromide, lithium chloride, lithium trifluoroacetate, lithium triflate of a lewis acid such as titanium isopropoxide in an organic solvent. The organic solvent used may be solvents such as, for example, dichloromethane, chloroform, toluene, benzene, tetrahydrofuran, dimethylformamide and the like or mixtures thereof. The reaction may be carried

out from room temperature to about 100 °C for 1-7 days to provide a compound of formula I, where residue A' is converted to residue A" having formula (i).

Step 2:



A", wherein X, Y are as defined

(i)

A, wherein X, Y are as defined

The compounds of formula I in an organic solvent, where residue A" has formula (i), are then subjected to standard hydrogenation conditions using a catalyst such as, but are not limited to, a catalytic amount of palladium on carbon in a hydrogen atmosphere to provide the saturated compounds of formula I, where in particular, residue A" having formula (i) is converted to residue A, as described throughout the specification.

The organic solvents used can be solvents such as methanol, ethanol, ethyl acetate or mixtures thereof. Other catalysts useful to assist hydrogenation may be, for example, but not limited to, platinum metal or its oxide [*see* standard books on catalytic hydrogenation, e.g., Rylander, P.N., *Hydrogenation Methods*, Academic Press: NY, 1985; *Catalytic Hydrogenation in Organic Synthesis*, Academic Press: NY, 1985; Červený, L., *Catalytic Hydrogenation*, Elsevier: NY, 1986 etc.]. The reaction may be carried out at room temperature or elevated temperature, for example, but not limited to, 50 °C or 100 °C.

Examples

The procedures described above for preparing the compounds of the present invention will be better understood in connection with the following examples, which are intentended to be illustrative only and not limiting of the scope of the invention. Various changes and modifications of the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications,

including without limitation, those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods fo the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

<u>Example 1: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.</u>

Cyclosporin methyl ester (0.030 mg, 0.024 mmol) and palladium on carbon (0.0012 mg, 0.0012 mmol) were added to a flask and the flask was evacuated and backfilled with hydrogen gas three times. Anhydrous methanol (3 ml) was added and the reaction was stirred for 18 h at ambient temperature under an atmosphere of hydrogen. After filtration and concentration in vacuo, the product was isolated as a white solid (0.021 mg, 70 % yield). Electrospray mass spectrum (ESMS) M+H: 1248.91

<u>Example 2: Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.</u>

The title compound of example 2 was prepared from cyclosporin ethyl ester and palladium on carbon according to the procedures described in Example 1. ESMS M+H: 1262.3

<u>Example 3: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.</u>

The title compound of example 3 was prepared from cyclosporin propyl ester and palladium on carbon according to the procedures described in Example 1.

<u>Example 4: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.</u>

The title compound of example 4 was prepared from cyclosporin benzyl ester and palladium on carbon according to the procedures described in Example 1.

<u>Example 5: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.</u>

The title compound of example 5 was prepared from cyclosporin fluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 6: Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 6 was prepared from cyclosporin difluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 7: Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 7 was prepared from cyclosporin trifluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1.

Example 8: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 8 was prepared from cyclosporin trifluoroethyl ester ester and palladium on carbon according to the procedures described in Example 1.

The cyclosporin analogs of the present invention have potent immunosuppressive and anti-inflammatory activity. In particular, they inhibit antigen-induced inflammatory cell infiltration, for example, into the airways. In vivo this activity is apparent following topical administration, e.g., pulmonary route.

The immunosuppressive and anti-inflammatory properties of cyclosporin analogs of the invention may be demonstrated in standard test models *in vitro* and *in vivo* for example as follows.

Example 9: Calcineurin Inhibition Assay

The immunosuppressive activity of cyclosporin is mediated through inhibition of the phosphatase activity of the enzyme calcineurin by a cyclophilin-cyclosporin complex. Thus, calcineurin inhibition is widely used as an *in vitro* measure of the activity of cyclosporin analogs.

Compounds were tested in an assay based on the Biomol Green Calcineurin Assay Kit supplied by Biomol (Plymouth Meeting, PA), supplemented with Cyclophilin A for enzyme inhibition. The activity of the recombinant human calcineurin was determined by release of phosphate from a phosphopeptide

representing a fragment of camp-dependent protein kinase. Phosphate release was determined using the colorimetric detection reagent Biomol Green (Biomol AK-111).

Compounds in DMSO (2.4μl) were added to a 96-well microplate and mixed with 50μl assay buffer (50mM Tris-HCl, pH 7.5; 100mM sodium chloride; 6mM magnesium chloride; 0.5mM dithiothreitol, 0.025% NP-40, 500μM calcium chloride, 0.27μM Calmodulin) containing 10μM Cyclophilin and 3nM Calcineurin. After warming to 37 °C for 60 mins, the enzymatic reaction was initiated by addition of phosphopeptide (7.5μl) to give a final concentration of 94μM. Phosphate release after 60 min at 37 °C was determined by addition of Biomol Green (100 μl) and measurement of the absorbance at 620nm after 15 mins at room temperature.

IC_{50} values were calculated from determinations of enzyme activity at inhibitor concentrations ranging from 0.1 to 0.0015 μM.

Example 10. NFAT reporter gene assay

NFAT activation follows precisely the activation of calcineurin by increased free calcium levels in the cytoplasm. Researchers from diverse fields are interested in the NFAT family of transcription factors, which are potential targets for newer and safer immunosuppressive drugs. In addition, the activation of NFAT proteins involves various cellular signal transduction pathways, including calcium mobilization and MAP kinase pathways linked to T-cell receptors and Ras1. To assist researchers probing the activity of NFAT proteins, Stratagene has developed a PathDetect cis-reporter plasmid, the pNFAT-Luc reporter plasmid (Stratagene, Inc. catalog # 219094), containing the NFAT binding site from the human IL-2 gene.2,7-9. The NFAT cis-reporting system includes the transfection-ready pNFAT-Luc reporter plasmid and the pCIS-CK negative control plasmid.

Construction of the pNFAT-Luc Plasmid:

The backbone of the 5749-base-pair pNFAT-Luc plasmid is the pFR-Luc reporter plasmid of the aforementioned PathDetect trans-reporting system. To this backbone, the GAL4 binding element was replaced with four direct repeats of the NFAT binding sequence (–286 to –257) from the IL-2 gene promoter, the most studied and widely used NFAT binding sequence. For all reporter plasmids of the PathDetect cis-reporting systems, activation of the luciferase gene indicated

interaction of uncharacterized gene products, extracellular stimuli, growth factors, or drug candidates with specific enhancer elements. Then a plasmid expressing the gene of interest was cotransfected into mammalian cells along with a cis-reporter plasmid to indicate transcription activation.

Testing the pNFAT-Luc Plasmid in Jurkat Cells:

Pharmacology studies have established that NFAT proteins can be activated by the protein kinase C activator phorbol ester (PMA) in combination with the calcium ionophore ionomycin, reagents that raise free intracellular calcium. When Jurkat cells, a mature human T-cell line, or CHO cells were transfected with the pNFAT-Luc plasmid and treated with 60 ng/ml of PMA and 1μg/ml of inomycin, luciferase activity increased by 13- and 16-fold, respectively. Therefore, the enhancer element in the pNFAT-Luc plasmid is responsive to calcium mobilization. Cells transfected with pNFAT-Luc and then treated with either PMA or ionomycin alone did not show a significant increase in luciferase activity.

Cyclosporin inhibits the activity of calcineurin, a protein phosphatase regulated by intracellular calcium mobilization. All the isoforms of NFAT protein contain a calcineurin-binding domain and are activated by calcineurin. The inhibition of luciferase expression from pNFAT-Luc in the present model, in both Jurkat and CHO cells induced by PMA and ionomycin, was monitored for cyclosporin (as a positive control) and the cyclosporin analogs of the present invention.

In another set of experiments, rat basophilic leukemia cells stably transfected with chemokine receptors were transfected with pNFAT-Luc and then treated with their respective ligands (data not shown). When both luciferase expression and calcium levels were monitored in these cells, luciferase expression correlated very well with calcium mobilization. Therefore, luciferase expression from pNFAT-Luc indeed reflects the activation of endogenous NFAT proteins by calcium immobilization.

Example 11. Immunosuppressive Activity and Applications

Murine Mixed Lymphocyte Reaction

Ca. $0.5x10^6$ lymphocytes from the spleen of female (8-10 weeks) Balb/c mice are incubated for 5 days in 0.2 ml cell growth medium with ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) CBA mice. Test substance is

added to the medium at various concentrations. Activity is assessed by ability to suppress proliferation-associated DNA synthesis as determined by incorporation of radiolabelled thymidine.

Mishell-Dutton Test

Ca. 10^7 lymphocytes from the spleen of OFI, female mice are co-cultured with ca. $3x10^7$ sheep erythrocytes for 3 days. Test substance is added to the incubation medium in varying concentrations. Lymphocytes are harvested and plated onto agar with fresh sheep erythrocytes as antigen. Sensitized lymphocytes secrete antibody that coats the erythrocytes, which lyse to form a plaque in the presence of complement. Activity is assessed by reduction in the number of plaque forming, i.e., antibody product, cells.

Delayed-type Hypersensitivity Resonse

On Day 0 groups of ten mice (having BALB/cByJ or any other acceptable strain) are dosed with test compound (1 to 10%), vehicle or the positive control, cyclophosphamide (Cyclosporin A), and monitored from Day-2 to 7. The mice are anesthetized and their abdomens shaved. 100 μl of a 3% solution of ovalbumin are applied to the abdomen and dried. Seven days later, the mice are challenged by applying 5 μl of ovalbumin to each side of the right ear. After 24 hours, both the right and left ear thickness are measured using a micrometer caliper.

Popliteal Lymph Node Assay

First, an inducer (phenytoin) is injected into the mice footpad (having BALB/cByJ or any other acceptable strain). Then the mice are challenged (subcutaneously or po) with ester and control agent using graded doses, for example, 2.5, 10, 20 mg/Kg (based on cyclosporine A data). On day 7 the popliteal lymph nodes are excised from the dosed mice and the lymph nodes are weighed. Then single cell suspensions of each lymph node are prepared and enumerated. The weight index for each animal is calculated (for example, a mean weight index <2 would indicate suppression of immune response).

Influence on Allergen-Induced Pulmonary Eosinophilia (*in vitro*)

Male Himalayan spotted guinea pigs (300 g, BRL) are sensitized to ovalbumin (OA) by i.p. injection of 1 ml of a suspension of OA (10 μg/ml) with $Al(OH)_3$ (100 mg) and B-pertussis vaccine (0.25 ml) in saline (0.9% w/v). For oral studies, the procedure is repeated 1x after 2 weeks and the animals are used one

week later. For inhalation studies, the procedure is repeated 2x at 3-week intervals and the animals are used one week after the last injection.

Challenge is effected employing a saline solution of OA, nebulized for discharge into an exposure chamber. Test animals are exposed to OA by nose-only inhalation for 60 minutes. For inhalation studies, OA solution is used at a concentration of 0.01%.

Test substance is administered (a) inhalation and/or (b) orally. For oral studies, test substance is administered p.o. in olive oil 1x daily for 3 days or in powder form in methylcellulose once prior to OA challenge. On day 3, test animals receive test substance 1.5 hrs. prior to and 6 hrs. after OA challenge. For inhalation studies, test substance is micronised for delivery to test animals restrained within a flow-past, nose-only inhalation chamber. Administration by inhalation is effected 15 mins. prior to OA challenge.

Efficacy of administered test substance is determined by bronchoalveolar lavage (BAL) and cell counting. For this purpose animals are sacrificed with Na pento-barbitone (100 mg/kg i.p.) and the trachea is exposed and cannulated. 5 successive 10 ml aliqots of Ca^2+ and Mg^2+ free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10mM) and HEPES (10 mM) is then introduced into the lung and immediately aspirated by gentle compression of the lung tissue. Total cell counts in pooled eluates are determined using an automatic cell counter. Lavage fluid is centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of supplemented HBSS. 10 μl of this cell suspension is added to 190 μl of Turk's solution (1:20) dilution). Differential cell counts are made from smears stained by Diff-Quick. Cells are identified and counted under oil immersion (x1,000). A minimum of 500 cells per smear are counted and the total population of each cell type is calculated.

In untreated animals, OA challenge induces increase of all cell types in BAL fluid 24 hours after challenge. Prior administration of cyclosporin analogs in accordance with the present invention by inhalation at dosages of the order of from 1.0 to 15.0 mg/kg reduces eosinophil count in BAL in a dose dependent manner as compared with untreated controls. Cell counts for other leucocytes (macrophages, neutrophils etc.) are also reduced.

Claims

What is claimed is:

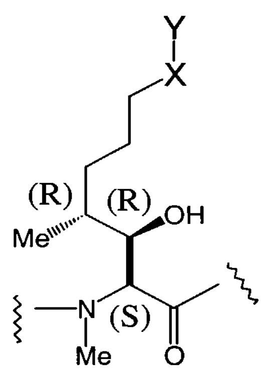
1. A cyclosporin analog of formula (I) or a pro-drug or a pharmaceutically acceptable salt thereof:

$$\boxed{\begin{array}{l} \text{A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal} \\ \;1\quad\;\, 2 \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad 8 \end{array}}$$

(I)

wherein,

(a) A is of the formula:



wherein

X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-;

Y is selected from the group consisting of:

i. -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

ii. -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

iii. -C(O)-OCH$_2$-OC(O)R2 where R2 is C1-C6 alkyl, optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio, heterocyclics or aryl;

iv. -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; and

v. C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio.

(b) B is –αAbu-, -Val-, -Thr- or –Nva-; and

(c) U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-; or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

2. A cyclosporin analog according to Claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, wherein in formula (I), B is -αAbu-, and U is -(D)Ala-.

3. A cyclosporin analog according to Claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, wherein in formula I:

(i) A is of the formula A1 or A2, wherein:

X is absent; and

Y is selected from a group consisting of:

i. -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio;

ii. -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; and

iii. C(O)-OCH_2-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio, heterocyclics or aryl;

(ii) B is -αAbu-; and

(iii) U is -(D)Ala-.

4. A cyclosporin analog according to claim 1 or a pro-drug or a pharmaceutically acceptable salt thereof, selected from the group consisting of:

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOH;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOEt;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2CH_2CH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2Ph$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2F$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCHF_2$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCF_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2CF_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2Cl$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2OCH_3$;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -$COOCH_2OCH_2CH_2O$ CH_3;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -C(=O)SCH_2Ph;

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is -$CH_2CH_2CH_2$-, Y = -$COOCH_3$; and

Compound of Formula (I) wherein B = –αAbu-, U = –(D)Ala-, X is absent, Y = -COOFmoc.

5. A chemical process for preparing a cyclosporin analog of formula I as claimed in Claim 1, comprising:
   a. reacting a compound of formula I, wherein A= -MeBmt- with:
      i. an olefin of formula CH_2=CH-X-Y, wherein X and Y are as defined in Claim 1; and
      ii. a catalyst;

      in the presence of a lithium salt in an organic solvent; and
   b. hydrogenating the product of step a in an organic solvent under hydrogen with a catalyst;

      and optionally converting the product of said reaction into a pharmaceutically acceptable salt.

6. The chemical process as claimed in Claim 5, wherein the catalyst in step (a) (ii) is Grubb's ruthenium alkylidene, Nolan's catalyst, a benzylidene catalyst or a molybdenum catalyst.

7. The chemical process as claimed in Claim 5, wherein step (b) is performed at room temperature.

8. The chemical process as claimed in Claim 7, wherein the catalyst in step (b) is Palladium on carbon.

9. A pharmaceutical composition, said composition comprising at least one cyclosporin analog of formula 1 as claimed in Claim 1, said cyclosporin analog being present alone or in combination with a pharmaceutically acceptable carrier or excipient.

10. A method for treating diseases characterized by airflow obstruction in a subject in need of treatment which comprises the step of administering to said subject a therapeutically effective amount of at least one cyclosporin analog of formula I as claimed in Claim 1.

11. The method of Claim 10, wherein said disease is asthma.

12. The method of Claim 10, wherein the step of administering the cyclosporin analog of formula I is done by topical administration.

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(54) Title: METHOD AND COMPOSITION FOR DRY EYE TREATMENT

(57) Abstract: A method and composition for treating a dry eye condition by topically applying to the eye surfaces an emulsion forming a tear film that acts to lubricate the eye and to inhibit evaporation therefrom. The emulsion is constituted by water in which is dispersed a mixture that includes a phospholipid, a non-polar oil, a non-toxic emulsifying agent and a polar lipid that imparts a net positive charge to the film that is distributed throughout the film, causing the film to be electrostatically attracted to the anionic surface of the eye whereby the film adheres thereto and cannot be washed away. Includable in the mixture is a non-soluble therapeutic agent, such as cyclosporin which is effective against an eye disease and is delivered to the eye by the film.

METHOD AND COMPOSITION FOR DRY EYE TREATMENT

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the treatment of a dry eye condition, and in particular to a method and composition for this purpose which forms an artificial tear film on the surface of the eye acting to inhibit evaporation therefrom and delivering to the eye surface an efficacious medicament to treat an eye disease.

STATUS OF PRIOR ART

The main concern of the present invention is with the treatment of a dry eye condition by a method and composition that acts to lubricate the eye and to reduce evaporation of fluid from the cornea surface. The cornea normally functions to maintain this surface in a moist and lubricated state which is impaired when the eye suffers from a dry eye condition.

Dehydration of moisture from the eye gives rise to various discomforts such an ocular dryness as well as burning and scratching sensations. But the more serious consequence of a dry eye condition is a loss of visual acuity which if it persists and is not corrected, may result in permanent damage. Dry eye disease acts to degrade the exposed ocular surface and may cause a complete breakdown of corneal tissues. In an extreme case, this may necessitate a corneal transplant.

Symptoms accompanying a dry eye condition are exacerbated when the eye is covered by a contact lens. The rate of evaporation of liquid from the eye is accelerated by the contact lens whose presence results in a meniscii formation that promotes evaporation even when the eye has an adequate natural tear film.

The usual treatment prescribed for a dry eye condition is to alleviate its symptoms by the topical application of a tear film substitute that adds a substantial

volume of liquid to the anterior surface of the eye. A typical composition functioning as a tear film substitute includes soluble polymer solutions. Of prior art interest in this regard is the US patent to Trager 4,421,740 which discloses an artificial tear composition formed by an aqueous hypotonic solution of lecithin, a phospholipid, and a viscosity-adjusting agent.

Of particular prior art interest are the following US patents in each of which Korb is a co-inventor. Hence these patents will hereinafter be referred to as Korb patents:

I. 4,914,088 (1990)
II. 5,278,151 (1994)
III. 5,371,108 (1994)
IV. 5,294,607 (1994)

The Korb patents point out that a normal eye has an ocular surface coated with a tear film composed of:

(a) a mucous inner layer in contact with the ocular surface of the eye

(b) an aqueous middle layer which is the source of moisture, and

(c) a lipid outer layer which minimizes evaporation of the moisture from the film.

"Dry eye" is experienced when the outer layer (c) of the tear film is defective. The dry eye treatment disclosed and claimed in Patents I to IV involves the topical application to the eye of phospholipids which form an artificial film over the eye that replicates a normal outer lipid layer and maintains the eye in moist condition.

Patent I is directed to an artificial tear film formed by:

"a layer of a complex phospholipid having a net positive or negative charge".

According to this Korb patent, the significance of a net positive or negative net charge is that in either case, the charged molecules in the film coating the surface of the eye *"repel each other"* and in doing so, maintain *"the integrity of the phospholipid therein"* so that it acts *"as a barrier reducing*

evaporation." Hence it is a negative or positive repelling charge that the inventor regards to be the crux of his invention.

Patent I fails to take into account that the surface of the eye being treated is anionic and therefore will interact electrostatically with a charged coating in a way that depends on the polarity of the charge. According to Patent I, the polarity of the charge doesn't matter, for in either polarity the charged molecules in the film repel each other.

An important aspect of the present invention is not only that it has a positive net charge, but also that the strength and distribution of the charge is such as to cause the film to adhere electrostatically to the entire anionically-charged eye surface to provide an effective moisture barrier. A weak positive charge would not achieve this result. Inasmuch as in present invention, the positively-charged molecules in the film covering the eye surface electrostatically engage the negatively-charged molecules on this surface, the resultant electrostatic couple is neutral and the couples do not repel each other.

Korb patent II discloses an eye treatment composition comprising

"a layer of a complex phospholipid having a net charge" and

"a layer of an essentially non-polar oil over said phospholipid layer", the phospholipid and oil layers being in an amount *"below that amount that would result in significant prolonged blurring of vision".*

According to Patent II, the preferred phospholipids are those "carrying a net negative charge because the negatively-charged molecules would be repelled by the negatively-charged ocular surface, thereby permitting the maintenance of a relatively thick aqueous layer".

In contradistinction, the present invention which resides in a positively-charged composition, exploits the fact that the eye surface is negatively charged (anionic) so that the composition is electrostatically attracted to this surface to create a coating which prevents the escape of moisture from the eye surface for a prolonged retention period.

Korb patent III also discloses a composition in which the phospholipid has a net negative or positive charge causing molecules in the tear film coating the eye surface to repel each other to maintain the integrity of the film. In Korb patent IV, the eye treated composition is a mixture of a charged phospholipid and a non-polar oil in a meta-stable water emulsion.

Essential to the present invention is that the emulsion coating the eye surface to form a film thereon carries a net positive charge which is distributed uniformly throughout the film so that it is electrostatically attracted to the entire anionic eye surface whereby the molecules on the film surface do not repel each other but are attracted to the eye surface.

Also of particular prior art interest is PCT patent publication WO 95/31211 (25 Nov. 1995) of Allergan, Inc. This publication discloses an emulsion for topical application to ocular tissue which includes cyclosporin admixed with castor oil. As noted in this publication, cyclosporin comprises a group of cyclic oligopeptides, the major component of which is cyclosporin A ($C_{62}H_{111}N_{11}O_{12}$), Clyclosporin has been found to be effective in the treatment of a dry eye condition.

SUMMARY OF THE INVENTION

In view of the foregoing, the main object of this invention is to provide an improved method and composition for treating a dry eye condition by topically applying to the eye surface an emulsion forming a tear film that adheres electrostatically to the entire surface of the eye and acts to lubricate the eye and to inhibit evaporation of moisture therefrom.

Among the significant advantages of a method and composition in accordance with the invention are the following:

A. The tear film derived from the emulsion carries a strong net positive charge that is uniformly distributed throughout the film surface whereby the film is electrostatically attracted to the entire area of the negatively-charged eye surface and there is no uncoated zone.

B. The electrostatic attraction between the artificial tear film and the eye surface maintains adhesive contact therebetween for a prolonged retention period and the tear film cannot be readily washed away.

C. The tear film coating the eye surface has no adverse effects, for the film includes no toxic or other harmful agents.

Also an object of this invention is to provide a composition of the above type which incorporates therein a therapeutic agent for treating an eye disease, such as cyclosporin A which when the composition is topically applied then delivers the agent to the eye. The release of the agent from the coating film to the surface of the eye is maintained for a prolonged period in that the film is held electrostatically in contact therewith.

Briefly stated, these objects are attained in a method and composition for treating a dry eye condition by topically applying to the eye surfaces an emulsion forming a tear film that acts to lubricate the eye and to inhibit evaporation therefrom. The emulsion is constituted by water in which is dispersed a mixture that includes a phospholipid, a non-polar oil and a polar lipid that imparts a net positive charge to the film that is distributed throughout the film, causing the film to be electrostatically attracted to the anionic surface of the eye whereby the film adheres to the eye and cannot be washed away. Includable in the mixture is a non-soluble therapeutic agent, such as cyclosporin which is effective against an eye disease and is delivered to the eye by the film.

DETAILED DESCRIPTION OF THE INVENTION

Cyclosporin A (CsA), a lipid-soluble cyclic endecapeptide, is a potent and well established immunomodulator drug mainly for oral use. With oral formulations, CsA bioavailability is limited because of the drug's insolubility in water and its tendency to separate immediately as a solid after coming into contact with water. Moreover, the bioavailability is highly dependent on complex

interactions occurring between the formulation and the physiological environment of the lumen.

CsA has been found to be effective in treating the immune-mediated keratoconjunctivitis sicca (KCS or dry eye disease) by the enhancement or restoration of lachrymal gland tearing in patient suffering from this syndrome. Dry eye disease is characterized by chronic drying of the conjunctiva and cornea, as well as by decreased tear production and changes in the composition of the tear film. In order to enhance the efficiency of CsA treatment, it becomes necessary to increase the absorption of the drug in the lachrymal gland as well as the conjunctiva and cornea target tissues, using for the purpose a suitable dosage of the drug to suppress ocular inflammation without significant systemic CsA exposure.

Since the aqueous solubility of CsA is between about 20 to 30 μg/ml, there is no adequate aqueous formulations available for ocular administration of the drug. Moreover, if cyclosporin is administered orally for the treatment of KCS, the accompanying side effects due to systemic circulation may cause adverse reactions such as hypertrichosis or renal dysfunction. In addition, the concentration of CsA present in oral formulations is limited due to the drug's hydrophobic nature.

Studies on ocular CsA penetration in animals were carried out using CsA formulations based on olive oil and corn oil. Local toxic effects on the cornea attributable to topical CsA formulations or the intrinsic solvent were observed. Upon using CsA in olive oil, in an *ex vivo* examination on bovine cornea, histological study revealed that the corneal epithelium was keratinized with some necrotic cells and rare pycnotic nuclei. Moreover, several researchers have confirmed that the probable toxic effect was due to topically administered CsA dissolved in olive oil. The conclusion reached is that olive oil, rather than CsA was responsible for the surface epithelial defects developing in the cornea. Hence, because of its high hydrophobicity, it is necessary to formulate CsA with compatible vehicles. These are not always biocompatible with ophthalmic administration, and may present some problems of stability such as the rancidity of olive oil. The drawback of corn-oil concentrated ointment formulations is that they

may exacerbate the symptoms (early-burning, redness and itching) of a dry eye condition.

It is possible to minimize problems related to unpleasant sensations and syndrome exacerbation by reducing the oil content and dispersing the oil phase in a water phase, resulting in an emulsion. We have found that when castor oil is used in developing the emulsion dosage, there are additional benefits to patients with dry eye disease arising from the long ocular retention time of the emulsion vehicle. The castor oil droplets in the emulsion form a lipid layer over the tear film, reducing the evaporation of the limited natural tears produced while the emulsion remains in the eye of a patient.

Our investigation of a positively-charged submicron emulsion containing a phospholipid having Zeta potential values ranging from 34 – 45 mV and a mean droplet size of around 150-250 nm supports the significant advantages which are gained when the emulsion vehicle carries a net-positive charge, rather than either a negative or neutral charge.

The resultant electrostatic attraction between the positively-charged submicron oil droplets in the emulsion and the corneal eye surface, which is negatively-charged results in a more prolonged residence or retention time conducive to topical drug flux enhancement.

Hence a positively-charged submicron emulsion of CsA enhances the local concentration of this medicament in conjunctiva and cornea which are the target ocular tissues. A positively-charged emulsion in accordance with the invention is therefore far more efficacious therapeutically than a negative charge emulsion having a similar composition.

The Composition The following represent formulations for a composition in accordance with the invention for treating a dry eye condition and other eye diseases.

Formulation (1) is a positive blank emulsion to be applied topically to an eye surface to create on the surface an artificial tear film. Formulation (2) which is for a CsA positive emulsion has the same ingredients as formulation (1), to which is

added cyclosporin. The resultant film serves as a vehicle to deliver the medicament to the eye surface.

Cyclosporin A	0.00	0.20
Castor oil	2.50	2.50
Lipoid E-80	0.50	0.50
Stearylamine	0.12	0.12
Vitamin E	0.01	0.01
Pluronic F-68	0.42	0.42
Glycerol	2.25	2.25
Benzalkonium chloride	0.01	0.01
Distilled water to	100.00	100.00

Lipid E-8 is a non-polar phospholipid, stearylamine is a cationic lipid and therefore imparts to the emulsion which also includes a non-polar castor oil a net positive charge. Pluronic F-68 is the trademark for poloxamer 188, a polyoxyalkyline derived from polypropylene glycol. Poloxamer 188 is an emulsifying agent and the glycerol in the formulation functions as an osmotic agent. Benzalkonium chloride is a cationic surfactant antiseptic agent acting as a preservative of the emulsion and strengthening the positive charge imparted to the emulsion by the cationic lipid. Vitamin E acts as a lipophillic antioxidant and as an eye lubricant.

In practice a composition may include instead of the cationic lipid stearylamine, cationic lipid oleylamine. The relative percentages of the ingredients included in the composition are not limited to those set forth above. Thus the relative percentage of castor oil may be in the range of 0.5 to 10%, that of the phospholipid (Lipoid E-80) in the range of 0.1 to 2.0%, that of the cationic lipid in the range of 0.1 to 0.5%, and that of the emulsifying agent, (Pluronic F-68), in the range of 0.5 to 2.0%.

It is vital however that whatever are the relative ranges of these ingredients, that the emulsion carry a net positive charge of sufficient strength to cause the emulsion when forming a film on the anionic surface of an eye, that it be electrostatically attracted to the surface so that it adheres thereto and cannot be readily washed away.

Lipoid E80, Pluronic F-68 and stearylamine coact to improve the stability of the emulsion droplets which are preferably in the submicron range, by enhancing the mechanical strength of the interfacial films formed around the droplets

It is important to bear in mind that in a composition in accordance with the invention which is to be administered topically to the anionic surface of an eye, that the phospholipid and castor oil included in the formulation carry no charge and that the aggregate net positive charge imparted to the submicron droplets is derived from the cationic surfactant plus the cationic antiseptic agent.

The advantage of this formulation over a dry eye treatment composition in which the charge imparted to the droplets is derived only from the phospholipid, as in the Korb patents, is that with the present formulation the positive charge of the emulsion is uniformly distributed over the entire area of the artificial tear film which is produced when the emulsion coats the anionic surface of the eye.

This results in electrostatic attraction throughout the entire area of the eye surface so that no portion thereof remains uncoated and untreated. Hence the present invention affords a treatment for a dry eye condition in which evaporation moisture is inhibited over the entire eye surface and no moisture is permitted to escape therefrom.

Preparation of Composition

Poloxamer 188 (Pluronic F-68) the osmotic agent (glycerol), and benzalkonium chloride were dissolved in the aqueous phase. The lipid E-80 is first dissolved in ethanol (1:5) and then dispersed in the aqueous phase. The ethanol is evaporated during the heating process of the aqueous phase. An antioxidant (α-tocopherol), the cationic lipid stearylamine (or oleylamine) and the CsA were dissolved in the castor oil phase. Both phases were heated separately to 70°C. The water phase was slowly incorporated into the oily phase and mixed with a magnetic stirrer. The resulting mixture was further heated to a temperature of 85°C.

The coarse emulsion obtained was emulsified for 5 minutes, using a high shear Polytron mixer and then rapidly cooled to below 20°C. After cooling in an ice bath, the emulsion was homogenized using a two stage homogenizer valve

assembly for 5 minutes. After further rapid cooling below 20°C, the pH was adjusted to 7.0 using 0.1 N hydrochloric acid. The emulsion was then filtered through a TE membrane filter (Schleicher & Schuell, Dassel, Germany) with a pore size of 0.45 μm. Finally, the emulsion was packed under nitrogen atmosphere in siliconized glass bottles and then sterilized by autoclaving at 121°C for 15 minutes. It is desirable that the droplets of the emulsion be in the submicron range and it is vital that the emulsion which is to be applied topically to the eye surface be sterile.

Medicaments: In an emulsion in accordance with the invention which is to be applied topically to the surface of an eye to treat a dry eye condition can also function as a vehicle to deliver a therapeutic agent to the eye to treat an eye disease.

The common practice in treating an eye infection is to deposit drops of an antibiotic agent in the eye, the number of drops to be applied on any one occasion being prescribed by a physician. Since this number defines the dosage of the drug applied to the eye, one must be careful that the drops are limited to the eye and that none of the applied liquid escapes therefrom. But in practice, it is difficult to deposit a drop of liquid into the eye so that none of the liquid flows beyond the eye borders, for there is little to hold the liquid to the eye surface.

The advantage of using an emulsion in accordance with the invention as a vehicle to deliver a therapeutic agent to the eye is that the emulsion which coats the entire surface of the eye and spreads the agent over its anionic surface, adheres electrostatically to this surface so that all of the therapeutic agent in a predetermined dosage is delivered to the eye. And because the coating electrostatically adheres to the eye surface and cannot be washed away, the residence time of treatment is prolonged and the therapeutic agent is therefore more effective.

The fact that the droplets in the charged emulsion in accordance with the invention are of submicron size is significant. This results in a much greater charge density per unit area of the emulsion film than would be produced had the droplet size been in the micron range and therefore produces a more powerful electrostatic force.

We have in the foregoing disclosed cyclosporin A as a preferred medicament to be incorporated in the emulsion. But other water-insoluble medicaments may be used provided that they possess properties for the treatment of eye disease similar to those of cyclosporin and are non-polar. Should the medicament carry a negative charge, then the amount of the cationic ingredient included in the emulsion must be such as to provide a net positive charge.

Thus among suitable medicaments that can be incorporated in an emulsion in accordance with the invention are those in the family of compounds including tacrolimus disclosed in US Patent 4,894,366. Also suitable is Sirolimus (Rapamycin) disclosed in US Patent 3,993,749.

While there has been disclosed preferred embodiments of the invention, it is to be understood that many changes may be made therein without departing from the spirit of the invention.

CLAIMS:

1. An emulsion to be topically applied to the anionic surface of an eye to form a tear film thereon which lubricates the eye to inhibit evaporation of fluid therefrom; said emulsion comprising:

A. water, and

B. a mixture dispersed in the water including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts a net positive charge to the tear film, causing it to be entrostatically attracted to the anionic eye surface and to adhere thereto to inhibit said evaporation

2. An emulsion as set forth in Claim 1, to treat a dry eye condition, the emulsion being defined by droplets in the submicron range.

3. An emulsion as set forth in Claim 1, in which the oil is castor oil.

4. An emulsion as set forth in Claim 1, in which the phospholipid is Lipoid E-80.

5. An emulsion as set forth in Claim 1, in which the cationic lipid is stearylamine.

6. An emulsion as set forth in Claim 1, in which the cationic lipid is oleylamine.

7. An emulsion as set forth in Claim 4, in which the relative percentage of the phospholipid in the emulsion lies in the range of 0.1 to 0.5 percent.

8. An emulsion as set forth in Claim 1, in which included in the mixture is vitamin E.

9. An emulsion as set forth in Claim 1, in which the mixture further includes an emulsifying agent.

10. An emulsion as set forth in Claim 9, in which the emulsifying agent is poloxamer.

11. An emulsion as set forth in Claim 10, in which the relative percentage of the emulsifying agent in the emulsion lies in the range of 0.5 to 2.0 percent.

12. An emulsion as set forth in Claim 1, in which the mixture further includes glycerol.

13. An emulsion as set forth in Claim 1, further including a cationic antiseptic agent.

14. An emulsion as set forth in Claim 1, in which the antiseptic agent is benzalkonium chloride.

15. An emulsion as set forth in Claim 1, in which the mixture further includes a water-insoluble medicament to treat eye disease.

16. An emulsion as set forth in Claim 15, in which the medicament is cyclosporin.

17. An emulsion as set forth in Claim 15, in which the medicament is tacrolimus.

18. An emulsion as set forth in Claim 15, in which the medicament is sirolimus.

19. A method of treating a dry eye condition comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

20. A method as set forth in Claim 19, in which the emulsion is prepared to create submicron droplets thereof.

21. A method as set forth in Claim 19, in which the mixture includes a water-insoluble medicament.

AMENDED CLAIMS

[Received by the International Bureau on 23 December 2002 (23.12.02) ; new claims 22-24 ; remaining claims unchanged]

12. An emulsion as set forth in Claim 1, in which the mixture further includes glycerol.

13. An emulsion as set forth in Claim 1, further including a cationic antiseptic agent.

14. An emulsion as set forth in Claim 1, in which the antiseptic agent is benzalkonium chloride.

15. An emulsion as set forth in Claim 1, in which the mixture further includes a water-insoluble medicament to treat eye disease.

16. An emulsion as set forth in Claim 15, in which the medicament is cyclosporin.

17. An emulsion as set forth in Claim 15, in which the medicament is tacrolimus.

18. An emulsion as set forth in Claim 15, in which the medicament is sirolimus.

19. A method of treating a dry eye condition comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

20. A method as set forth in Claim 19, in which the emulsion is prepared to create submicron droplets thereof.

21. A method as set forth in Claim 19, in which the mixture includes a water-insoluble medicament.

22. A method of treating immune-mediated keratoconjunctivitis sicca comprising the steps of:

A. preparing an emulsion in which water has dispersed therein a mixture including a non-polar phospholipid, a non-polar oil, a

non-toxic emulsifying agent and a cationic lipid which imparts to the emulsion a net positive charge, and further includes cyclosporin A; and

B. topically applying the emulsion to an eye surface to form a tear film which is electrostatically attracted to the anionic surface of the eye whereby the film adheres to the surface.

23. Use of an emulsion for the preparation of a pharmaceutical composition for the treatment of a dry eye condition, said emulsion comprising:

A. water, and

B. a mixture dispersed in the water including a non-polar phospholipid, a non-polar oil, a non-toxic emulsifying agent and a cationic lipid which imparts a net positive charge to the tear film, causing it to be entrostatically attracted to the anionic eye surface and to adhere thereto to inhibit said evaporation

24. The use according to Claim 23 for the preparation of a pharmaceutical composition for the treatment of immune-mediated keratoconjunctivitis sicca, wherein said emulsion further comprises cyclosporin A.

Statement under Article 19(1)

New Claim 23 is a reformulation of claim 19 in a form acceptable to the European Patent Office. New claims 22 and 24 claim a particular embodiment of the invention and find support in the specification on page 5, lines 3-11 and page 7, lines 20-24.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 01/01015

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/107 A61K38/13 A61K31/436 A61P27/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 18852 A (YISSAM RESEARCH DEV COMPANY OF) 30 September 1993 (1993-09-30) page 1, line 2 - line 6 examples 1-14,16 claims 1,3,18 ---	1,2, 4-12,15, 19-21

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2002	23/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Epskamp, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 01/01015

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	ABDULRAZIK M ET AL: "Effect of emulsion droplet surface charge on cyclosporine ocular tissue distribution." IOVS, vol. 42, no. 4, 15 March 2001 (2001-03-15), page S925 XP008005391 Annual Meeting of the Association for Research in Vision and Ophthalmology;Fort Lauderdale, Florida, USA; April 29-May 04, 2001 abstract	1-21
X	KLANG SH ET AL: "Physiochemical characterization and acute toxicity evaluation of a positively-charged submicron emulsion vehicle" JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 46, no. 12, 1994, pages 986-993, XP008005426 ISSN: 0022-3573	1,2,4,5, 7-12,15, 19-21
Y	abstract page 986, left-hand column, line 1 - line 9 page 987, left-hand column, last line -right-hand column, paragraph 3 page 991, left-hand column, paragraph 3 -right-hand column, paragraph 1 page 992, left-hand column, paragraph 3	1-21
X	KLANG SH ET AL: "Evaluation of a positively charged submicron emulsion of piroxicam on the rabbit corneum healing process following alkali burn" JOURNAL OF CONTROLLED RELEASE, vol. 57, no. 1, 1999, pages 19-27, XP004155636 ISSN: 0168-3659	1,2,4,5, 7-12,15, 19-21
Y	abstract paragraph ‘02.2! paragraph ‘0004!	1-21
X	KLANG S ET AL: "Influence of emulsion droplet surface charge on indomethacin ocular tissue distribution" PHARMACEUTICAL DEVELOPMENT AND TECHNOLOGY, vol. 5, no. 4, 2000, pages 521-532, XP008005503 ISSN: 1083-7450	1,2,4,5, 7-12,15, 19-21
Y	abstract page 522, right-hand column, last paragraph -page 523, left-hand column, paragraph 2 page 531, left-hand column, paragraph 2	1-21

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 01/01015

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 02 09667 A (PHARMASOL GMBH) 7 February 2002 (2002-02-07) page 19, line 1 - line 5; examples 22-24 -----	1,2,4,5, 7-12,15, 16,19-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 01/01015

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 19-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 01/01015

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9318852	A	30-09-1993	IL 101241	A	20-11-1997
			AT 182485	T	15-08-1999
			AU 670443	B2	18-07-1996
			AU 4368393	A	21-10-1993
			CA 2132210	A1	30-09-1993
			DE 69325796	D1	02-09-1999
			DE 69325796	T2	09-03-2000
			DK 630286	T3	29-11-1999
			EP 0630286	A1	28-12-1994
			ES 2134850	T3	16-10-1999
			GR 3031623	T3	31-01-2000
			JP 7504848	T	01-06-1995
			WO 9318852	A1	30-09-1993
			US 6007826	A	28-12-1999
WO 0209667	A	07-02-2002	DE 10036871	A1	14-02-2002
			AU 8976801	A	13-02-2002
			BR 0107042	A	04-06-2002
			WO 0209667	A2	07-02-2002

Form PCT/ISA/210 (patent family annex) (July 1992)

Ocular delivery of cyclosporin A
II. Effect of submicron emulsion's surface charge on ocular distribution of topical cyclosporin A

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The aim of the present work is to compare and to gain useful mechanistical understanding of the effect of surface charges of the emulsion droplets on topical cyclosporin A penetration through the various ocular tissues. Following one single 50 µl dose into the rabbit eye, it was found that the submicron emulsion vehicle bearing a positive charge enhanced cyclosporin A bioavailability in the conjunctiva and cornea compared to the negatively-charged emulsion. This is probably due to the interaction between the positive charge of the emulsion vehicle surface and the negative charge of the corneal surface at physiological pH. Such an electrostatic interaction was already demonstrated by published ex vivo and in vivo studies and is supported by the present data. The enhanced penetration of CsA in the ocular tissue is apparently mediated by an endocytic mechanism, the occurrence of which can be confirmed by the intermediate results of unpublished cell-culture studies. Nevertheless, the cyclosporin A concentration in the intraocular tissues was low indicating poor drug penetration. In addition, there was no marked drug concentration in the various untreated eye tissues, indicating the lesser contralateral effects exerted by the emulsion dosage forms, irrespective of the droplet surface charge.

Key words: Topical cyclosporin A – Positively-charged emulsion – Conjunctiva – Cornea – Absorption – Ocular surface charge – Ocular pharmacokinetics of CsA.

New ophthalmic-controlled drug delivery systems (hydrophilic matrix, soluble polymers, Ocusert, gels) have been developed [1-3] in an attempt to circumvent the well-known low ocular bioavailability of topically-instilled drugs [4]. There is still a need to develop a suitable drug delivery system not only for improving the ocular bioavailability of the topically-instilled drug but also for enhancing drug absorption into target ocular tissue.

Cyclosporin A (CsA), a lipid-soluble cyclic endecapeptide, is a potent and well-established immunomodulator drug mainly for oral use. With oral formulations, CsA bioavailability is limited because of the insolubility of the drug in water and its tendency to separate immediately as a solid on contact with water. Furthermore, CsA absorption in the gastrointestinal tract can be erratic from one formulation batch to the next, requiring constant monitoring of drug blood levels during treatment.

A novel, negatively-charged emulsion of CsA was recently developed and clinically tested for the treatment of immuno-inflammatory eye diseases such as *keratoconjunctivitis sicca* (KCS or dry eye syndrome) [5]. The CsA emulsion proved to be effective, suggesting local as opposed to systemic action. Ocular CsA therefore represents a significant step forward in the treatment of patients with KCS. Previous agents were strictly palliative in nature, but CsA emulsion may represent the first therapeutic agent with a mechanism of action targeted at the underlying immune-mediated inflammatory response that occurs in patients with KCS [5]. In a randomized, multicenter, double-masked, parallel-group, dose response-controlled Phase 2 clinical study conducted more recently by Stevenson *et al.* [6] on human subjects, it was observed that ophthalmic, negatively-charged emulsions (exhibiting zeta potential values of - 50 mV) containing different CsA concentrations of 0.05, 0.1, 0.2, and 0.4% were safe, well tolerated and significantly improved the ocular signs and symptoms of moderate to severe dry eye disease. In another published report [7], the same group observed in a Phase 3 study with a similar dose response-controlled clinical trial that the novel ophthalmic formulations of CsA at concentrations of 0.05 and 0.1% were safe and effective in the treatment of moderate to severe dry eye disease yielding improvements in both objective and subjective measures. However, despite these encouraging clinical results, the promising novel, negatively-charged emulsion has not yet been approved by the FDA because statistically significant efficacy parameters in the two Phase 3 trials were not replicated between studies and therefore, additional clinical studies were requested.

A positively-charged submicron emulsion was reported to be effective as an ocular delivery system for a variety of drugs [8-10]. The rationale to design a novel submicron emulsion vehicle bearing a positive charge instead of either a negative or neutral charge should be sought in the potential interaction occurring between the positively-charged emulsion droplets and the corneal surface, which is negatively charged at physiological pH [11]. The data reported in the literature suggest a possible electrostatic mechanism resulting in a prolonged residence time of the positively-charged emulsion on the negatively-charged corneal surface.

Kiang *et al.* [10] have reported the potential of a positively-charged submicron emulsion suitable for the ocular application of piroxicam. The piroxicam positively-charged emulsion was

seen to be the most effective formulation in lowering the ulcerative cornea score following alkali burn of rabbit corneas. An increased residence time of the positively-charged emulsion droplets on the negatively-charged corneal surface is a plausible explanation for the resulting enhancement of lipophilic drug ocular disposition. More recently, in another *ex vivo* study [8], the positively-charged emulsion was also noted to have better wettability properties on the corneal surface compared to either saline or the negatively-charged emulsion. Furthermore, the spreading coefficient of the positively-charged emulsion on the cornea is four times greater than that of the negatively-charged emulsion. Laboratory animal studies also confirmed that the positively-charged emulsion enhanced the corneal bioavailability of indomethacin [8].

The aim of the present work is therefore to develop and characterize a positively-charged ophthalmic emulsion containing CsA and to compare the penetration potential of CsA into the different ocular tissues from positively- and negatively-charged submicron emulsions. It is also intended to promote a useful mechanical understanding of the enhancing ocular penetration effect of the submicron emulsion as a function of the vehicle surface charge.

I. MATERIALS AND METHODS

1. Materials

CsA was provided by Galena, a.s. (Czech Republic) and met European Pharmacopoeia specifications. Castor oil was purchased from Fluka and complied with European Pharmacopoeia specifications. Lipoid E-80 was purchased from Lipoid GmbH (Ludwigshafen, Germany). According to the manufacturer's specifications, Lipoid E-80 contained about 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% non-polar lipids, and about 2% sphingomyelin. Poloxamer 188 (Pluronic F-68) was supplied by BASF (Parsippany, NJ, United States). Stearylamine and α-tocopherol were purchased from Sigma (MO, United States). All the other ingredients used were of pharmaceutical grade.

2. Methods

2.1. Emulsion preparation and characterization

The CsA-loaded positively- and negatively-charged emulsions were prepared according to the method described in Part 1 of this study published herewith. Briefly, hydrophilic excipients were dissolved in the aqueous phase whereas the lipophilic excipients were dissolved in the lipophilic phase with the exception of Lipoid E-80, which was first dissolved in ethanol (1/5) and then dispersed in the aqueous phase. Both phases were heated separately to 70°C, then mixed and stirred with a magnetic stirrer. The resulting mixture was further heated to 85°C. At this temperature, the resulting coarse emulsion was further mixed for 5 min using a Polytron high-shear mixer and then rapidly cooled to below 20°C. After cooling, the emulsion was homogenized at 9000 lb.in^{-2} for 5 min using a two-stage homogenizer valve assembly and then again rapidly cooled to below 20°C. The pH of the emulsion was adjusted to 7 using 0.1 N hydrochloric acid. The emulsion was then filtered through a TE membrane filter (Schleicher & Schuell, Dassel, Germany) with a pore size of 0.45 µm. The emulsion was packed under nitrogen atmosphere in glass vials and then sterilized at 121°C for 15 min in a steam autoclave. The emulsion was stored at 4°C over prolonged periods of time. The typical positively-charged emulsion formulation consists of (w/w%): CsA (0.2), castor oil (2.5), Poloxamer 188 (0.425), glycerol (2.25), Lipoid E-80 (0.5), stearylamine (0.12), α-tocopherol (0.01), benzalkonium chloride (0.01) and water (up to 100). The negatively-charged emulsion formulation contains the same ingredients but instead of 0.12% stearylamine, 0.2% deoxycholic acid is added. The produced emulsions were characterized physicochemically (zeta potential, pH, main droplet diameter, and drug content) as per the standard methods stated in Part 1 of this study.

2.2. Cyclosporin radio-labelled formulations

Radio-labelled CsA emulsions were prepared by adding 10 µl of ^{3}H- cyclosporin A ethanol solution (1 mCi/ml, with a specific activity of 7.0 Ci/mmole supplied by Amersham, Buckinghamshire, United Kingdom) to 1 ml of various cyclosporin formulations; the mixture was then stirred overnight. The radio-labelled emulsion was tested for stability, appearance, homogeneity, pH, total CsA and radioactivity counts before and after dosing.

3. Animal experiment study

This investigation adhered to the Hadassah University Hospital Ethical Committee resolution on the use of animals in research (Declaration of Helsinki, the Guiding Principles on the Care and Use of Animals, DHEW publication, NIH 80-23S).

3.1. *In vivo* ocular distribution in rabbits

White albino rabbits (2-2.5 kg) were separated randomly into two groups, and each group (N=18) was treated with either positively- or negatively-charged radio-labelled 0.2% CsA-loaded submicron emulsions, respectively. One drop (50 µl) was instilled into the right eye (*cul-de-sac*), then three rabbits were sacrificed at selected time points (15, 30, 60, 120, 180 and 480 min). The right eyes were enucleated and dissected. The cornea, conjunctiva, aqueous humor, iris, lens, vitreous, sclera-choroid-retina and venous blood samples were weighed before storage in preweighed vials and frozen (- 80°C) until analysis. Radioactivity was determined by combustion of [^{3}H] cyclosporin in a sample oxidizer (model 307, Packard Instrument Co., Meriden, CT, United States) and liquid scintillation counting. The final drug quantity was calculated per gram for ocular tissues and per millilitre for aqueous humor and blood.

3.2. Unilateral and contralateral effects in rabbit eyes

The time point at which optimal drug levels were achieved in the conjunctiva after dosing with the emulsions was chosen for studying the bioavailability of CsA in the contralateral eye. One drop (50 µl) was instilled into the right eye (*cul-de-sac*). At 60 min post-dose, the drug-treated right eye as well as the untreated left eye were enucleated and dissected. The CsA concentration in the various tissue was determined by the method described above. In this particular part of the study, in addition to the previously mentioned ocular tissues, the optic

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nerve was also dissected and subjected to the same experimental conditions as the other ocular tissues for CsA determination.

3.3. Statistical analysis

Statistical analysis of the drug concentrations and the area under the curve (AUC) in the animal experiments was carried out using χ^2 test.

II. RESULTS

Table I displays the various physicochemical properties and stability assessment of positively- and negatively-charged submicron emulsions containing 0.2% w/w of CsA. The absolute zeta potential value was high for both emulsions. A similar droplet size distribution was obtained with an average diameter in the range of 220-240 nm for both type of emulsions.

Figures 1 and 2 show the CsA concentration change over time in the various ocular tissues (cornea, conjunctiva, iris and sclera-choroid-retina) up to 8 h after one single instillation of 50 µl of drug-loaded positively- and negatively-charged emulsions in the *cul-de-sac* of rabbit eyes, respectively. Significant differences were observed in the CsA concentrations in the various ocular tissues between the groups treated with the positively- and negatively-charged emulsions. When compared to the negatively-charged emulsion, the positively-charged emulsion elicited significantly higher drug concentrations in the cornea and conjunctiva at each of the tested time periods ($p<0.05$), except at 180 min. At this test time, the drug concentration in the conjunctiva elicited by negatively-charged emulsion seems to be higher than the positively-charged one. In contrast, in the iris, both positively- and negatively-charged emulsions elicited almost the same drug concentrations at all times tested. In the case of sclera-choroid-retina, there was no difference in behavior between the positively- and negatively-charged emulsions during the initial time periods. However, at 60 and 120 min, the drug concentration resulting from the positively-charged emulsion was notably higher than that of the negatively-charged emulsion, and no differences in the concentrations were noted in the other time periods. At 60 and 120 min, the CsA concentration increased from 0.14 ± 0.16 to 0.25 ± 0.30 µg/g and from 0.04 ± 0.01 to 0.1 ± 0.05 µg/g, respectively ($p<0.05$). It should also be noted from the data depicted in *Figures 1 and 2* that the CsA concentrations in the cornea and conjunctiva fluctuate while substantial concentrations can still be measured in these tissues after 8 h, indicating the existence of a reservoir effect in the ocular surface tissues especially with the positively-charged emulsion.

The values of the various pharmacokinetic parameters are shown in *Table II*. Maximum CsA concentrations and AUC_{0-8h} values, reflecting the extent of ocular absorption (bioavailability), were reached in the conjunctiva followed by the cornea, iris and sclera-choroid-retina. Both parameters were significantly higher for the positively-charged emulsion than for the negatively-charged emulsion in the conjunctiva and cornea ($p<0.05$) (*Table II*). It should be noted that, irrespective of the emulsion charge, all other intraocular tissues such as the lens, vitreous and

Table I - Characterization and stability assessment of the cyclosporin A-loaded submicron emulsions.

Property	Negatively-charged emulsion	Positively-charged emulsion
Actual drug content (% w/w)	0.198	0.205
Initial droplet diameter (nm ± sd)	235 ± 52	220 ± 48
Zeta potential (mV)	- 40	+ 60.5
Drug content after 6 month storage at 4 C	*	0.195

* The prolonged stability was not followed up for negatively-charged emulsion as it was not stable after few weeks storage at 4 C.

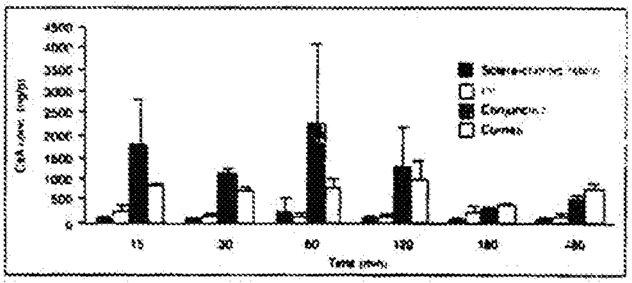

Figure 1 - Effect of positive surface charge on cyclosporin A (CsA) concentration in extraocular tissues following single instillation of 50 µl of CsA-submicron emulsion in the *cul-de-sac* of rabbit eye.

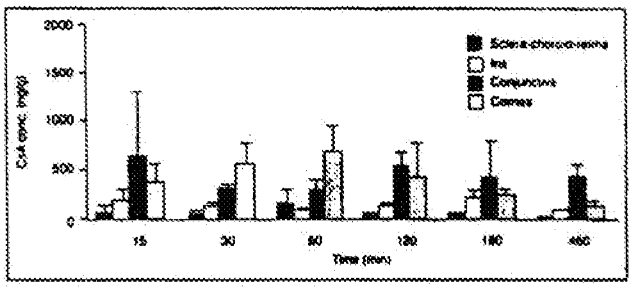

Figure 2 - Effect of negative surface charge on cyclosporin A (CsA) concentration in extraocular tissues following single instillation of 50 µl of CsA-submicron emulsion in the *cul-de-sac* of rabbit eye.

Table II - Ocular pharmacokinetics parameters obtained following single instillation of 50 µl CsA- loaded submicron emulsions in rabbit eyes

Tissues	T_{max} (min)		C_{max} (µg/g)		AUC_{0-8h} (µg·min·g^{-1})	
	Emuls(-)*	Emuls(+)**	Emuls(-)*	Emuls(+)**	Emuls(-)*	Emuls(+)**
Cornea	60	120	0.68 ± 0.30	1.02 ± 0.44***	143.1	328.95***
Conjunctiva	15	60	0.64 ± 0.67	2.29 ± 1.78***	206.18	373.58***
Iris	180	180	0.22 ± 0.09	0.25 ± 0.16	70.43	90.53
Sclera-choroid-retina	60	60	0.14 ± 0.16	0.25 ± 0.30***	23.78	44.63

* Negatively-charged submicron emulsion. ** Positively-charged submicron emulsion. *** p < 0.05.

PTO-000451

blood, with the exception of the aqueous humor, elicited low CsA levels (*Figures 3 and 4*). Furthermore, with exception of the aqueous humor, the maximum drug concentration in the various other intraocular tissues was less than 5% of that detected in the conjunctiva. Moreover, these tissues did not show any marked difference in drug concentrations and even the levels obtained were too low to consider any interpretation suggesting a low CsA penetration. Although significant, high therapeutic levels of CsA were found in the ocular rabbit tissues of interest (conjunctiva and cornea) after dosing with positively- and negatively-charged emulsions, less drug was absorbed systemically, as evident from the blood drug concentrations obtained after instillation of the dosage forms (*Figures 3 and 4*).

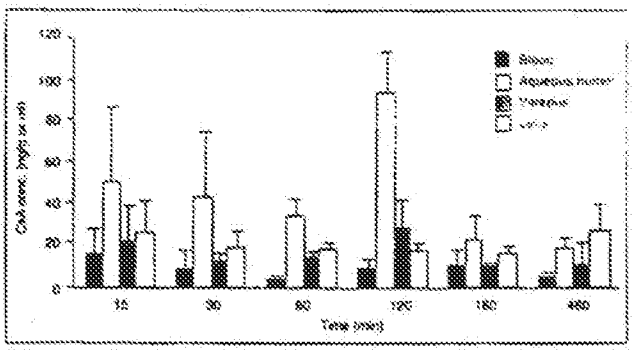

Figure 3 - Effect of positive surface charge on cyclosporin A (CsA) concentration in intraocular tissues following single instillation of 50 µl of CsA-submicron emulsion in the *cul-de-sac* of rabbit eye.

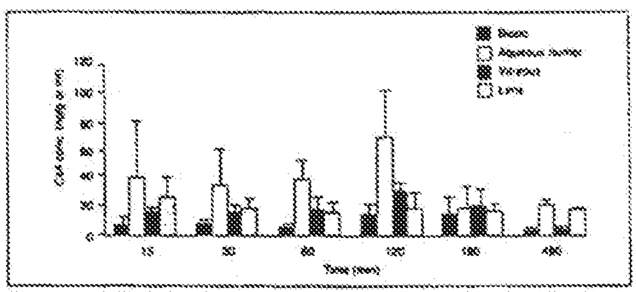

Figure 4 - Effect of negative surface charge on cyclosporin (CsA) concentration in intraocular tissues following single instillation of 50 µl of CsA-submicron emulsion in the *cul-de-sac* of rabbit eye.

To evaluate the contralateral effect of the present formulations, a repeated study was performed with an interval of 60 min post-instillation. *Figures 5 and 6* summarize the observations noted with negatively- and positively-charged emulsions in the contralateral (untreated) and unilateral (treated) eyes, respectively. Regardless of the surface charge of the emulsions, the treated rabbit eye showed a marked drug concentration in the cornea and conjunctiva of the eye (*Figure 6*), confirming the results of previous animal studies (*Figures 1 and 2*).

On the other hand, both formulations elicited detectable but negligible concentrations in the contralateral eye as depicted in *Figure 5* where the highest level reached for the conjunctiva was less than 6 ng/g. A very interesting finding to be observed closely from *Figure 6* is the level of CsA in the optic nerve. It can be seen that the concentration of CsA in the optic nerve

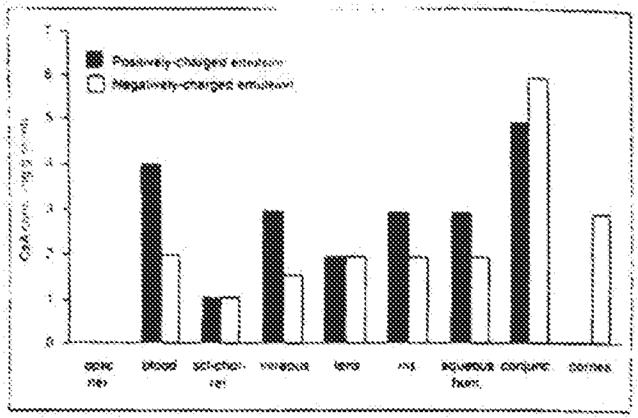

Figure 5 - Cyclosporin A (CsA) concentration in different ocular tissues after 60 min (from instillation of the submicron emulsions) in the untreated eye as a function of surface charge droplet.

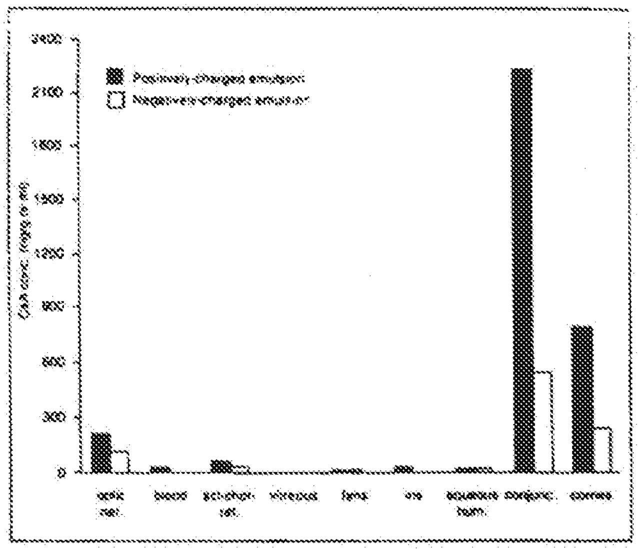

Figure 6 - Cyclosporin A (CsA) concentration in different ocular tissues after 60 min (from instillation of the submicron emulsions) in the treated eye as a function of surface charge droplet.

following instillation with the positively-charged emulsion is much higher than that recorded with the negatively-charged emulsion (*Figure 6*).

III. DISCUSSION

CsA-loaded negatively- and positively-charged emulsions were prepared following the well-established combined emulsification technique for sizing to the submicron range to yield fine stable homogeneous emulsions as displayed in *Table I*. CsA was found to be chemically stable in the stored positively-charged emulsion over six months at 4°C. Radio-labelling of emulsion using ^{3}H-cyclosporin A ethanol solution did not induce virtually any nanoprecipitation problem which indicates, therefore, that the behavior of the labelled CsA emulsions did not differ from that of ordinary emulsions. In addition, Acheampong *et al.* [5] found that tritium-labelled CsA is stable in the emulsion throughout the entire study. They clearly demonstrated that there was no metabolite in any of the ocular tissue analyzed. Consequently, they have deduced that the

concentration of radioactivity measured in the ocular tissue reflected an equivalent concentration of non-metabolized CsA, which should stand for the present study.

After a single dose of the positively-charged emulsion in the rabbit eye, CsA was markedly absorbed, yielding higher C_{max} of 2.29 ± 1.78 and 1.02 ± 0.44 µg/g in the conjunctiva and cornea, respectively, than with the negatively-charged emulsion, which yielded values of 0.64 ± 0.67 and 0.68 ± 0.30 µg/g, respectively (*Table II*). The difference between conjunctival and corneal C_{max} can probably be explained by the extent of and difference in surface area between these two ocular tissues. Assuming that the epithelial surface of the conjunctiva has the same negative charge as that of the corneal epithelial surface at physiological pH, the electrostatic mechanism will be more pronounced in the high surface area of the conjunctiva compared to the corneal surface area [11, 12]. In the case of the negatively-charged emulsion-treated ocular surface, the assumed repulsion effect between the negatively-charged vehicle surface and the negatively-charged ocular surface will be more pronounced with the high conjunctival surface area. This will partially explain the slightly lower CsA concentration (C_{max}) obtained in the conjunctiva compared to the cornea, because other factors like the higher conjunctival surface is available for absorption of the drug compared to the corneal surface.

Regardless of the ocular surface tissue, the positively-charged emulsion yielded the highest ocular bioavailability as reflected by the marked significant increase in AUC_{0-8h} values compared to the AUC_{0-8h} values yielded by the negatively-charged emulsions (*Table II*). For instance, the AUC values for the conjunctiva and cornea were increased by 180 and 230%, respectively. Since the CsA concentration is kept constant in both formulations (0.2%w/w), the significantly high drug concentrations observed in the conjunctiva and cornea could be directly attributed to the anticipated influence of the emulsion surface charge on potential drug penetration through the ocular surface tissues such as the conjunctiva and cornea. Although the CsA concentrations in the conjunctiva and cornea appeared to fluctuate, the fluctuations observed with the present emulsions do not differ from those results already reported in the literature. Wiederholt *et al.* [13] reported a biphasic profile of CsA in ocular tissues following single instillation (10 µl) of a 2% castor oil solution into the rabbit eye. The castor oil droplets in an emulsion may form a lipid layer over the tear film, possibly reducing the evaporation of the limited natural tears produced by these patients while the emulsion remains in the eye [5].

In addition, the ocular surface seems to serve not only as a barrier to drug penetration but also as a reservoir for CsA since the therapeutic concentration level was nevertheless maintained in the conjunctiva and cornea even after completion of the study at 480 min following positively-charged emulsion instillation (*Figure 1*). It should also be noted that the negatively-charged emulsion maintained a therapeutic level at 480 min, only in the conjunctiva (0.45 ± 0.12 µg/g) and not in the cornea (*Figure 2*) since the CsA cornea level was 0.16 ± 0.04 µg/g as compared to 0.84 ± 0.12 µg/g for the positively-charged emulsion. Furthermore, because of its high molecular weight, CsA should poorly permeate through the tight junction epithelial surface of the cornea. The fact that high levels of drug were detected in the cornea whilst the aqueous humor concentration was relatively low indicates that an endocytic mechanism of penetration is likely to occur, rendering the corneal epithelial layer a drug reservoir. It should be noted that the C_{max} of both the cornea and aqueous humor is reached at the same time (T_{max} = 120 min) but the CsA concentration in the aqueous humor is still significantly lower than that in the cornea, thus confirming the previous statement (1.02 ± 0.44 µg/g and 0.09 ± 0.02 µg/ml, respectively). This is also supported by the well-known stomal resistance to the diffusion of lipophilic drugs and confirms the results on CsA corneal penetration published by other authors [13, 14]. The reservoir effect, as observed after instillation of the positively-charged emulsion, has important clinical consequences since, following an initial loading dose, the ocular surface tissues may act to supply a therapeutic quantity of drug to other tissues over an extended period of time.

The findings of the present study are consistent with those observed previously with indomethacin positively-charged emulsion [8] and mainly with the results published on the negatively-charged emulsion of CsA [5]. After single dosing with this negatively-charged emulsion in the rabbit eye, CsA was rapidly absorbed yielding a C_{max} of 1.34 and 0.955 µg/g in the conjunctiva and cornea, respectively. The conjunctiva is considered to be one of the major target tissues in the treatment of dry eye [15]. Topical ophthalmic CsA therefore penetrated the conjunctiva at concentrations adequate for local immuno-modulation while penetration into intraocular tissues was far less marked and absorption into the blood was minimal [5].

A remarkable fact arising from the results of the present study is that the positively-charged emulsion provided higher CsA levels than the negatively-charged emulsion formulation. There is proof that colloidal delivery systems can facilitate the penetration of drugs into ocular surface tissues via an endocytic mechanism [16]. As previously suggested, the endocytic effect is probably more pronounced with the positively-charged emulsion. In experiments of keratocyte cell-cultures exposed to either negatively- or positively-charged blank emulsion, only the positively-charged oil droplets were internalized in the keratocytic cells (unpublished data). The assumed ocular surface tissue reservoir effect of positively-charged emulsion is also corroborated by the high drug levels observed in the optic nerve (*Figure 6*). Such high concentrations can be reached only by CsA diffusion through one of the following pathways: transcorneal, transconjunctival or through the blood circulation secondary to the systemic absorption. Since aqueous humor and blood CsA levels were found to be very low, only the transconjunctival route can represent a plausible approach culminating in such optic nerve concentrations in eyes treated with the positively-charged emulsion.

Although it is a well-known fact that the instillation of relatively large drops will lead to lesser ocular drug bioavailability [17], in the current study, a particularly large drop size dose (50 µl) was selected and kept constant irrespective of the experimental conditions in order to maximize the potential for systemic exposure as adverse effects associated with systemic CsA (hypertrichosis, renal dysfunction) appear to be dose-dependent [18]. Despite such large instilled doses, low CsA blood concentrations were observed suggesting that the clinical

use of castor oil-based positively-charged emulsion is unlikely to lead to any of the adverse effects associated with the systemic administration of CsA.

In spite of differences in blink frequency, ocular surface permeability and aqueous humor dynamics between rabbit and man, a mean CsA concentration of 236 ± 42 ng/g was reported for explanted human corneas (obtained from patients receiving corneal transplants) following topical dosing with 2% CsA in an olive oil formulation [19]. These data indicate that extrapolation of the results of animal studies to humans cannot be ruled out. Thus, topical administration of CsA-loaded positively-charged emulsion to patients with dry eye disease should provide adequate levels of CsA in ocular surface tissues to modulate the local immune response without significant systemic exposure.

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* *

In conclusion, the positively-charged emulsion vehicle was found to be more effective for delivering CsA to the ocular surface tissues than the negatively-charged emulsion at an identical dose level and time period. However, in order to optimize ocular drug delivery, it is imperative to understand and to establish the mechanism of positively- and negatively-charged emulsions-cell interactions at all possible ocular absorptive sites such as the cornea, conjunctiva and sclera. Such cell culture studies are currently underway and from the preliminary results, it can be concluded that CsA can be preferentially internalized in the target cells of the conjunctiva and cornea with the positively-charged emulsion. Furthermore, the positively-charged submicron emulsion yielded CsA levels in the target conjunctiva and cornea tissues well above the minimum therapeutic range (200-800 ng/g). It can therefore be anticipated that a significant local immunomodulation effect can be elicited by the positively-charged emulsion in the extraocular tissues while penetration into intraocular tissues was much less and absorption into the blood was minimal. It can be deduced that the CsA-loaded positively-charged emulsion may be superior in treatment of dry eye disease.

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CYCLOSPORINE DISTRIBUTION INTO THE CONJUNCTIVA, CORNEA, LACRIMAL GLAND AND SYSTEMIC BLOOD FOLLOWING TOPICAL DOSING OF CYCLOSPORINE TO RABBIT, DOG AND HUMAN EYES.

Andrew Acheampong, Martha Shackleton, Steve Lam, Patrick Rudewicz and Diane Tang-Liu. Allergan, Irvine, CA, USA.

Cyclosporine (CsA) is a selective and reversible inhibitor of T-lymphocyte actions. It offers potential therapy for dry eye disease in dogs and humans by inhibiting ocular surface immunoinflammatory processes. The topical route was selected to achieve therapeutic concentrations in the eye while avoiding the side effects of systemic CsA. The purpose of this report was to compare CsA concentrations in the ocular tissues or whole blood after topical application of CsA ophthalmic emulsion to rabbits, dogs and humans.

NZ albino rabbits received a single eyedrop of the 0.05%, 0.2% or 0.4% ^{3}H-CsA emulsion. CsA 0.2% emulsion eyedrops were applied to beagle dog eyes for 7 days. The total radioactivity concentration in the animal ocular tissues and blood collected over 0-96 hr intervals after the last dose were measured by LSC. In a Phase II clinical study, blood samples were collected throughout treatment period from dry eye patients dosed twice daily with 0.05%, 0.1%, 0.2% or 0.4% emulsion eyedrops for 3 months. The human blood concentrations were measured with a highly sensitive LC-MS/MS assay.

In the dogs given 0.2% emulsion, the rank order for the maximal mean tissue radioactivity concentration (in ng-CsA equivalents/g) was: tears (289000), conjunctiva (2010), cornea (1810), lacrimal gland (357) and blood (1.15). In the rabbits dosed with 0.2% emulsion, the C_{max} distribution was: tears (279000) cornea (1340), conjunctiva (955), lacrimal gland (9.80) and blood (0.745). Topical dosing in humans with 0.05%-0.4% CsA emulsion resulted in blood concentrations of less than 0.2 ng/ml. In a Sandoz study, human corneal concentrations (measured by RIA) were 4660 ± 4010 ng/g (mean ± SD, n = 117) after two doses of 2% CsA ointment were administered prior to surgery to "high risk" corneal transplantation patients. The ocular tissue concentrations in the rabbits were dose-dependent, with cornea and conjunctiva concentrations increasing less than proportional with dose.

In conclusion, topically applied CsA emulsion can produce therapeutic concentrations in the cornea, conjunctiva, and lacrimal gland at doses lower than the 2% CsA topical doses used in animal and human pharmacokinetic studies reported in the literature. Systemic blood CsA concentrations in humans, rabbits and dogs after topical CsA doses of the emulsions were much lower than the blood trough concentrations of 50-100 ng/ml used for monitoring therapeutic levels in systemic organ transplantation.

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CYCLOSPORINE DISTRIBUTION INTO THE CONJUNCTIVA, CORNEA, LACRIMAL GLAND, AND SYSTEMIC BLOOD FOLLOWING TOPICAL DOSING OF CYCLOSPORINE TO RABBIT, DOG, AND HUMAN EYES

Andrew Acheampong, Martha Shackleton, Steve Lam, Patrick Rudewicz, and Diane Tang-Liu

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1. INTRODUCTION

Cyclosporine is an immune modulator that inhibits T-lymphocyte-mediated immunoreactivity. Allergan is currently evaluating the clinical efficacy of 0.05%-0.4% cyclosporine emulsion for the treatment of immuno-inflammatory eye diseases, such as keratoconjunctivitis sicca, or dry eye syndrome. Topical ocular application of cyclosporine, formulated as 2% cyclosporine in olive oil, 0.2% cyclosporine in corn oil ointment (Schering-Plough), or 0.2% cyclosporine emulsion (Allergan), was found to reduce ocular surface inflammation and improve lacrimal gland secretion in dogs with KCS.[1-3]

The aim of the present research was to determine the ocular tissue distribution of cyclosporine in rabbits and dogs, and to compare tissue concentrations in rabbits, dogs, and humans after topical administration. Determination of relationships between the ocular tissue drug concentrations and efficacy is important for optimizing delivery of pharmacologically active concentrations in the target ocular surface tissues, providing support to the local mechanism of action, and optimizing dosing regimen.

2. METHODS

2.1. Animal Studies

[Mebmt -^{3}H]-cyclosporin-A was prepared by Amersham (UK) with radiochemical purity greater than 98%. Female New Zealand white rabbits (2–3 kg) received a single 50

μl dose of 0.2% ^{3}H-cyclosporine formulation (~1 mCi/ml) into the lower conjunctival cul-de-sac of the left eye. Male beagle dogs (10–13 kg) received a 35 μl dose of 0.2% ^{3}H-cyclosporine emulsion (~1 mCi/ml) into the lower conjunctival cul-de-sac, twice daily for 7 days. Ocular tissues and systemic blood were also collected at selected time points over a 96-h period postdose. Two dogs or four rabbits were used per time point. The rabbit experiments were conducted according to USDA and Allergan ACUC guidelines. The dog study was conducted at Huntingdon Life Sciences. Tissue radioactivity concentrations were expressed as ng equivalents (eq) of cyclosporine per gram of tissue, using the specific activity of the dose formulation.

2.2. Human Range-Finding Study

One hundred sixty-two human subjects with KCS received an eyedrop of vehicle or 0.05%, 0.1%, 0.2%, or 0.4% cyclosporine emulsion twice daily for 12 weeks. Blood samples were collected from all subjects at morning troughs after 1, 4, and 12 weeks of dosing. In addition, blood samples were collected from selected subjects at 1, 2, and 4 h after the last dose at week 12. Cyclosporin A (CsA) concentrations in blood samples were measured by a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method with Cyclosporin G as the internal standard. The lower limit of quantitation of the blood assay was 0.1 ng/ml.

3. RESULTS AND DISCUSSION

Figs. 1 and 2 depict the time course of cyclosporine in tears, ocular surface tissues, and orbital lacrimal gland of rabbits and dogs after eyedrop instillation of 0.2% ^{3}H-cyclosporine emulsion. Significant cyclosporine concentrations (C_{max}, ~1000 ng/g) were found in the conjunctiva and cornea, the target tissues for CsA reduction of ocular surface inflammation. The 0.2% emulsion provided approximately 7-fold higher cyclosporine concentrations in the rabbit cornea and conjunctiva than those for 0.2% cyclosporine in pure castor oil.[4] The lacrimal gland C_{max} was several-fold that of blood (~1 ng-eq/g), especially in the dog.

The ocular absorption and disposition of cyclosporine in rabbits and dogs were characterized by rapid absorption into ocular and extraocular tissues, reservoir effect of the cornea, relatively low intraocular tissue concentrations, and a long terminal elimination half-life of 20–44 h in most ocular tissues (Figs. 1 and 2). Similar ocular distribution characteristics were noted in previous rabbit and human studies.[4–7]

Table 1 shows less than 0.2 ng/ml blood concentrations in humans following multiple topical instillation of 0.05%, 0.1%, 0.2%, and 0.4% cyclosporine ophthalmic emulsion over a 12-week period of dosing. The systemic blood CsA concentrations in humans after topical CsA doses of the emulsions were much lower than the blood trough concentrations of 20–100 ng/ml used for monitoring the safety of patients receiving systemic cyclosporine therapy.

4. CONCLUSIONS

Topically applied cyclosporine emulsion can produce significant concentrations in the cornea and conjunctiva to exert a local immunomodulatory effect. The ocular distribu-

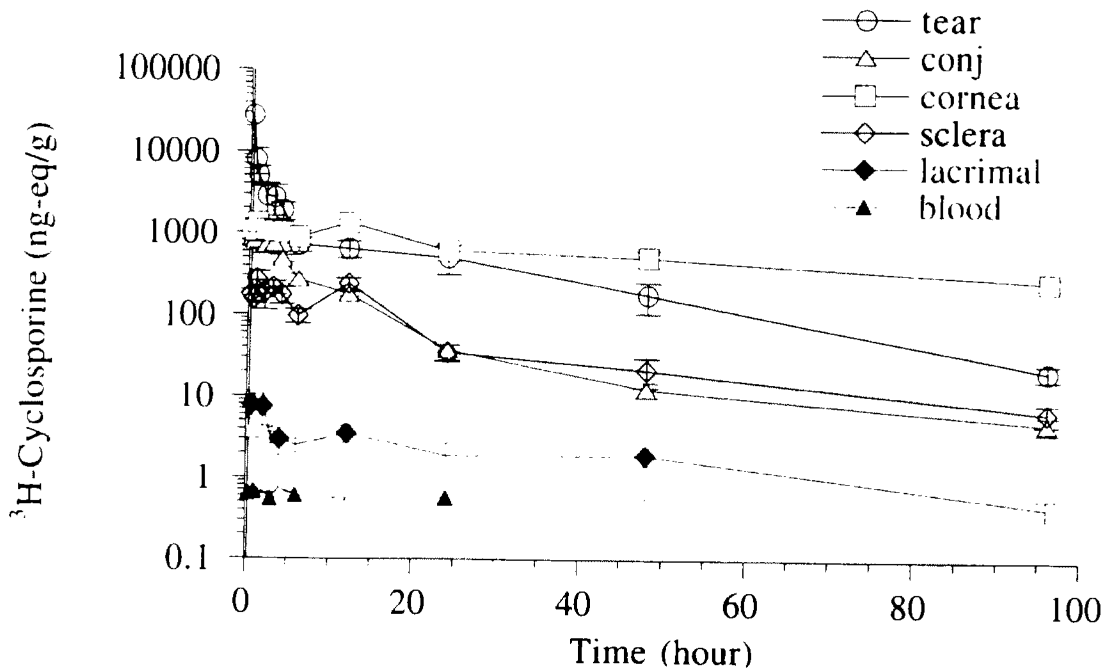

Figure 1. Total radioactivity concentrations (mean ± SEM) in rabbit eyes and systemic blood.

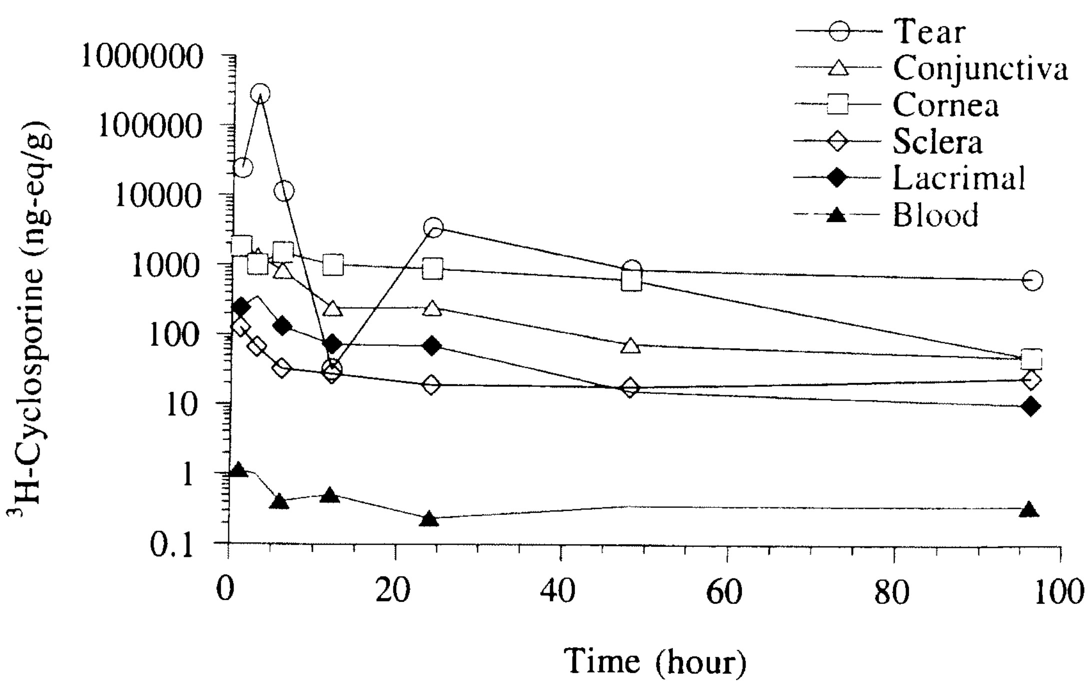

Figure 2. Total radioactivity concentrations (mean values) in dog eyes and systemic blood.

Table 1. Human blood trough and maximum cyclosporin A concentrations over 12 weeks

Cyclosporine emulsion	Range of blood cyclosporine A concentration (ng/ml)	
	Trough level	Maximum level
0.05%	<0.1	<0.1
0.1%	<0.1 to 0.102	<0.1
0.2%	<0.1 to 0.108	<0.1 to 0.144
0.4%	<0.1 to 0.157	<0.1 to 0.158

tion of cyclosporine after topical application of CsA emulsion was generally similar in rabbit and dog. In the rabbits dosed with 0.2% emulsion, the C_{max} tissue distribution was: tears > cornea > conjunctiva > lacrimal gland > blood.

Systemic blood cyclosporine concentrations following topical application of cyclosporine emulsion were very low in rabbits, dogs, and humans, obviating concerns about systemic toxicity or systemic mechanism of action. The human blood cyclosporin A concentrations were less than 0.2 ng/ml, much lower than the blood trough concentrations of 20–100 ng/ml used for monitoring the safety of patients receiving systemic cyclosporine therapy.

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Distribution of cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs

Andrew A. Acheampong, Martha Shackleton, Diane D-S. Tang-Liu, Shulin Ding, Mike E. Stern and Robert Decker

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Abstract

Purpose. To determine the ocular pharmacokinetics of cyclosporin A after topical ophthalmic administration.

Methods. Radiolabled cyclosporin A in either a castor oil-in-water emulsion or a corn oil ointment was applied to the eyes of beagle dogs or albino rabbits using the following paradigms: (i) single doses of 0.2% emulsion to rabbits and dogs, (ii) single doses of 0.05%, 0.2%, or 0.4% emulsion to rabbits, (iii) multiple doses of 0.2% emulsion to dogs, (iv) single and multiple doses of 0.2% ointment to rabbits. The distribution of cyclosporin A was determined by measuring the distribution of radioactivity.

Results. After a single dose, cyclosporin A was rapidly absorbed into the conjunctiva (C_{max}: dogs, 1490 ng/g; rabbits, 1340 ng/g) and cornea (C_{max}: dogs, 311 ng/g; rabbits, 955 ng/g). High concentrations (>300 ng/g) could be detected in the cornea up to 96 hours post-dose. Lower concentrations were found in the intraocular tissues, and systemic absorption was minimal. After multiple doses, there was some accumulation in the cornea, lens, lacrimal gland, and iris-cilliary body, but limited accumulation in the conjunctiva and sclera. Ocular tissue concentrations of cyclosporin A increased with increasing dose concentration; proportionally in lacrimal gland and intraocular tissues; less than proportionally in conjunctiva and cornea. The pharmacokinetic profile of the cyclosporin A corn oil ointment was similar to that of the emulsion.

Conclusions. Topical ophthalmic cyclosporin A penetrated into extraocular tissues at concentrations adequate for local immunomodulation while penetration into intraocular tissues was much less and absorption into the blood was minimal.

Keywords: *cyclosporin*; *ocular distribution*; dry eye; ophthalmic; pharmacokinetics; *topical administration*

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Introduction

Dry eye disease (also known as keratoconjunctivitis sicca) is characterized by the chronic drying of the sclera and conjunctiva, as well as by decreased tear production and changes in the composition of the tear film. Dry eye disease has recently been redefined as a localized inflammatory/autoimmune disease (1). Evidence suggests that a T-cell-mediated inflammatory response in the lacrimal gland acini and ducts contributes to the pathology of this condition (2-4). In addition, lymphocytic infiltration of the lacrimal gland has been associated with fibrosis, atrophy, and duct pathology in the lacrimal gland (2).

This new understanding of the pathophysiology of dry eye disease has promoted the use of the immunomodulatory drug, cyclosporin A, as a candidate therapy directed at the underlying inflammatory/autoimmune nature of the disease. When administered to patients with dry eye disease, cyclosporin A should suppress the immunoinflammatory processes on the ocular surface and in the main and accessory lacrimal glands, as well as enhance lacrimal secretion. Evidence for the latter comes from renal allograft recipients who experience increased tear flow – even in the absence of a deficit in baseline tear production – after systemic administration of cyclosporin A (5). Other clinical data show that cyclosporin A therapy can benefit other ocular inflammatory diseases such as severe vernal keratoconjunctivitis (6), primary Sjögren's syndrome (7), and secondary Sjögren's syndrome (8). In addition, cyclosporin A has been successfully used to prevent the rejection of corneal transplants (9, 10).

Further evidence to support the clinical use of cyclosporin A in dry eye disease comes from studies in beagle dogs that develop a spontaneous keratoconjunctivitis sicca-like syndrome. Topical administration of cyclosporin A (in either olive oil or corn oil) to affected dogs increased tear production as well as induced the regression of corneal neovascularization and granulation (11). Similarly, after topical administration of cyclosporin A in olive oil, Olivero *et al.* (12), observed clinical benefits in 81% of cyclosporin A-treated dogs with spontaneous

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keratoconjunctivitis sicca. The efficacy of cyclosporin A against the ocular surface after topical dosing suggests a local rather than a systemic action.

Integrated through a neuronal feedback loop, the ocular surface and the lacrimal glands are thought to function as a single unit. Since the lacrimal glands, upper conjunctiva, and cornea are the tissues affected in dry eye disease, the ocular surface is the ideal target for the topical administration of cyclosporin A in order to suppress ocular inflammation without significant systemic cyclosporin A exposure.

The poor solubility of cyclosporin A in aqueous solutions and the slow partition rate of cyclosporin A from oily vehicles into the corneal epithelium, has presented a challenge in preparing stable eyedrop formulations that can both provide therapeutic concentrations of cyclosporin A to the target ocular tissues and which have an acceptable safety profile. The hydrophobic nature of cyclosporin A should, however, permit it to penetrate into the corneal epithelium but not the hydrophilic stroma, resulting in relatively high corneal concentrations with relatively low aqueous humor concentrations (9). Laibovitz *et al.* (13) reported encouraging results when cyclosporin A in a corn-oil ointment was administered to patients with dry eye disease; however, early-burning, redness, and itching were associated with this particular formulation.

Animal safety studies have demonstrated that topical cyclosporin A formulations ranging in concentration from 0.05% to 1% are generally safe, with no systemic adverse effects and only mild transient ocular discomfort in some animals at higher concentrations (14,15). Preclinical studies of the ocular penetration of cyclosporin A in rabbit eyes have used formulations of cyclosporin A based in either olive oil (16) or castor oil (17). Studies in dogs have used either olive oil (11, 12) or corn oil (11).

The present report describes a series of studies which examined the ocular absorption, distribution, and elimination of cyclosporin A after its topical administration in a castor oil-in water emulsion or a corn oil ointment to both albino rabbits and beagle dogs. Preliminary results from these studies have been reported in abstract form at the Association of Research in Vision and Ophthalmology Conference (18) and the Second International Conference on the Lacrimal Gland, Tear Film, and Dry Eye Syndromes in 1996 (19).

Materials and methods

Reagents

All solvents were of HPLC grade and, unless otherwise stated, all other chemicals and reagents were of analytical grade.

Test substances

Cyclosporin A (also known as cyclosporine; 99% purity) was obtained from Novartis (Basel, Switzerland). β-^{3}H-cyclosporin A was prepared by Amersham (Buckinghamshire, England) by introducing the ^{3}H-label at a metabolically stable position in amino acid number 1 (methyl butenylmethyl threonine; (4R)-4-(E)-2-butenyl-4N-dimethyl-3-(^{3}H)-L-threonine). This material which is, hereafter, described as ^{3}H-cyclosporin A (^{3}H-CsA), had a radiochemical purity of 98% (as determined by reversed phase high pressure liquid chromatography [HPLC]) and a specific activity of 2.6 Ci/mmol (2.16 mCi/mg). A 1 mCi/mL solution was supplied in ethanol.

An ophthalmic ^{3}H-CsA castor oil-in-water emulsion (hereafter referred to as the emulsion) was prepared in a castor oil-in-water vehicle as previously described (20). The concentration of castor oil in the emulsion (expressed as % weight/weight [% w/w]) increased proportionally with the concentration of ^{3}H-cyclosporin A. The radiolabeled emulsion was tested for stability, appearance, homogeneity, pH, total cyclosporin A and radioactivity concentrations before and after dosing. Osmolality and oil globule size were determined to be identical to a non-radiolabeled formulation of cyclosporin A.

Also tested was a cyclosporine corn oil ointment (Novartis Pharmaceutical Corp., East Hanover, NJ).

Animals

Rabbits

Male and female adult New Zealand albino rabbits (2 to 3 kg) were obtained from Hazleton Research Products (Kalamazoo, MI) and, upon arrival were placed in quarantine for at least 7 days. Rabbits were randomly assigned to groups for subsequent ocular tissue collection. Food and water were supplied *ad libitum* and the lighting was controlled to provide 12 hours of light and 12 hours of darkness throughout the study.

Dogs

Male beagle dogs (approximately 6 to 7 months of age; 9.7 to 12.9 kg) were selected from stock animals held at Huntington Life Sciences (Huntington, England) and were originally obtained from Interfauna Ltd. (Wytin, U.K.). The dogs were fed a normal laboratory diet (SQC Lab Diet A, SDS Biosure, Withamn, Essex, UK). Drinking water was provided *ad libitum* throughout the study.

Administration of ^{3}H-Cyclosporin A

Drops of ^{3}H-cyclosporin A emulsion were instilled into the eyes of albino rabbits as follows. The lower eyelid was gently pulled away from the eye globe and, using a calibrated positive displacement pipettor, 50 µL of the ophthalmic emulsion was applied to the lower conjunctival cul-de-sac. After dosing, the upper and lower eyelids were hand-held together for approximately 5 seconds to permit the ^{3}H-cyclosporin A to come in contact with the cornea. The eye or eyes selected for dosing varied depending on the specific experimental protocol, as is described under *in vivo experimental procedures* below.

For dose administration to dogs in the pharmacokinetic studies, the animals were manually restrained and the lower

Table 1. Summary of pharmacokinetic studies

Formulation	Species	Dosing	Dose	Dose administration
Emulsion	Albino rabbits Beagle dogs	Single	0.2%	rabbits: 50 µL in left eye dogs: 35 µL in both eyes
Emulsion	Albino rabbits	Single	0.05%, 0.2%, 0.4%	50 µL in both eyes
Emulsion	Beagle dogs	Multiple	0.2%	35 µL in both eyes twice daily for 7 days
Corn oil ointment	Albino rabbits	Single and multiple	0.2%	Single: 20 µL in both eyes Multiple: 20 µL in both eyes once daily for 7 days

eyelid of each eye was gently pulled away from the eye globe. The emulsion (35 µL) was pipetted into the lower cul-de-sac of each eye. The upper and lower eyelids were hand-held closed for 30 seconds to allow the drug to come in contact with the cornea and the eyelids were then released. Any spillage of the dosing solution was collected by a cotton-tipped applicator and the radioactivity in each cotton tip was measured by Liquid Scintillation Counting (LSC) as described below.

The corn oil ointment was administered in a 20 µL volume to both eyes of rabbits using a calibrated pipette.

In vivo experimental procedures

Pharmacokinetics studies

A summary of the experiments examining the penetration and distribution of ^{3}H-CsA in ocular tissues is provided in Table 1. The pharmacokinetics of ^{3}H-CsA following single doses of the 0.2% emulsion were examined in both rabbits and dogs. The effect of multiple dosing on ^{3}H-CsA pharmacokinetics was studied in beagle dogs only, and the effect of increasing ^{3}H-CsA concentration was studied in albino rabbits. The pharmacokinetics of ^{3}H-CsA following single and multiple doses of the 0.2% corn oil ointment was examined in albino rabbits.

In the single-dose emulsion study in rabbits, animals were dosed in the left eye only. Four females per time point were sacrificed for tissue collection at 20 and 40 minutes, and 1, 2, 4, 6, 12, 24, 48, and 96 hours after dosing and four males per time point were sacrificed at 40 minutes, and 2 and 24 hours after dosing. Ocular tissues from both eyes of 2 untreated male and 2 untreated female rabbits were used as controls. In the single- and multiple-dose emulsion studies in dogs, animals were dosed in both eyes and 1 animal was sacrificed 1, 3, 6, 12, 24, 48, and 96 hours after the final dose. In the dose-response emulsion study in rabbits, animals were dosed in the left eye only and 3 animals per time point were sacrificed at 20 minutes and 1, 3, 6, 8, and 24 hours after dosing. In the studies using cyclosporin A ointment, animals were dosed in both eyes and 3 animals per time point were sacrificed at 20 minutes and 1, 2, 4, 6, 12, 24, 48 and 96 hours after dosing.

Prior to sacrifice, tear samples were collected from all animals onto a pre-tared Schirmer strip (Iolab Pharmaceutical, Claremont, CA) and weighed before carefully rinsing the eye with 2 mL of normal saline (0.9% NaCl). Immediately after tear sampling, blood was collected in an EDTA-treated vacutainer tube (2 mL from the marginal ear vein or artery of rabbits and 5 mL from the jugular vein of dogs). Ocular tissues were recovered from euthanized animals (rabbits received Eutha-6 [0.5 to 1.0 mL; Western Supply Co, Arcadia, CA]; dogs received pentobarbital sodium [150 g/kg]). Each tissue was dissected into very small pieces which were extracted twice with methanol. Aliquots of methanol extracts from plasma, aqueous humor, and vitreous humor were analyzed by LSC (Beckman 1801 scintillation counter, [Beckman, Fullerton, CA]) to measure the total radioactivity extracted. Samples of other ocular tissues and whole blood were combusted using a tissue oxidizer (Model 307 Packard Instruments, Downers Grove, IL). After combustion, $^3H_20$ was trapped by Monophase-S (Packard Instruments) and the radioactivity counted by LSC.

Ocular retention study

Infometric analysis was used to evaluate the ocular retention time (ORT) of cyclosporin A emulsion or cyclosporin A vehicle emulsion after topical instillation of a single drop to the eyes of beagle dogs. Since the castor oil content in the vehicle increases with the concentration of cyclosporin A, it was necessary to also test the vehicles for a range of cyclosporin A concentrations.

In all experiments, a baseline tear film examination was carried out prior to administration of the test material. Then, one drop of test material was instilled superiorly onto the corneal surface of the left eye. Observations of precorneal area were made at 3, 20, 60, 120, and 240 minutes post instillation. At the end of this observation period (4 hours post-dose), both eyes of each beagle were rinsed copiously with Lens Plus Rewetter® drops (Allergan, Inc. Irvine, CA). Tear evaluations were carried out using the Guillon interferometric technique as previously described (21).

Ocular metabolism of cyclosporin A

The reference standards for cyclosporin A metabolites were produced using in vitro liver cultures. Rabbit liver homogenates were prepared as previously described (22) and incubated with ^{3}H-CsA. The retention times of metabolites of

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^{3}H-CsA were characterized by HPLC analysis under conditions described previously (23, 24).

Fifty-microliters of the ^{3}H-CsA 0.2% emulsion were topically administered to the left eyes of a total of 14 rabbits (7 male and 7 female). Animals were sacrificed at 2, 6, and 24 hours after dosing. Ocular tissues from both eyes of an untreated male and an untreated female were used as controls. The total radioactivity present in ocular tissues samples, expressed as dpm/g or dpm/mL, was determined as described above. The percent recovery of total radioactivity in tissue extracts was calculated from the ratio of radioactivity in the extract to total radioactivity in the tissues. The percentage of peak area of either radiolabeled cyclosporin A or metabolite in the radiochromatograms of ocular tissue extracts were used to access the proportion of radiolabeled cyclosporin A and metabolites in the ocular tissues.

Data analysis and statistical methods

In both dog and rabbit, C_{max} in each tissue is defined as the highest concentration of ^{3}H-CsA and is taken directly from experimental data. The area under the concentration/time curve (AUC) from time zero to the last quantifiable time point (AUC_{0-T}) was calculated according to a linear trapezoidal method.

In the rabbit, the elimination rate constant (K_e) was determined from the last 3 to 5 time points in the terminal elimination phase. The terminal half-life ($T_{1/2}$) of the decline phase was calculated as K_e/0.693. With the sole exception of the tears, tissue concentrations at time zero were set to zero; in the case of tears from treated eyes, the concentration at time zero was estimated from the equation below [2].

[tear (0 hr)] = dose in ng/{rabbit tear weight + dose weight} [2]

In the dog, rate constants for the decline in radioactivity concentrations during the study period were determined by the method of residuals ("feathering").

The tissue concentration data was analyzed using Excel software (version 4.0, Microsoft Corp, Redmond, WA). Briefly, total radioactivity concentrations in whole blood and tissue samples were expressed in dpm/g or dpm/mL. These values were subsequently converted to ng equivalents (eq) of cyclosporin A /g or /mL using the formula below:

$$\text{ng-eq cyclosporin A/g tissue} = \frac{\text{dpm of sample / sample weight in grams}}{\text{(specific activity in dpm/nq-eq cyclosporin A)}}$$

Statistical evaluation of significant differences ($\alpha = 0.05$) between sample means by a two-tailed Student's t-test was made using a Primer statistical software (version 1.0, McGraw Hill, Inc., New York, NY). All data are presented as mean ± standard deviation unless stated otherwise.

Results

Ocular metabolism of cyclosporin A

The extent to which ocular tissues metabolize cyclosporin A was measured after topical administration of ^{3}H-CsA 0.2% emulsion to albino rabbits.

Radiochromatograms from the gradient HPLC analysis of each ocular tissue were compared with those obtained from liver homogenates that had been incubated with ^{3}H-CsA. Over the 23-hour period after administration of ^{3}H-CsA, the major radiolabeled material present in all ocular tissues examined was determined to be non-metabolized ^{3}H-CsA; there were no measurable levels of any metabolite in any of the tissues analyzed (data not shown).

In the experiments described below, the distribution of ^{3}H-CsA was measured in different ocular tissues after topical administration. Radiolabeled material was recovered with high efficiency from all ocular tissues tested (recovery values > 92%, Table 2). Consequently, the concentration of radioactivity measured in the ocular tissue was used to reflect equivalent concentrations of non-metabolized cyclosporin A (expressed as ng-eq /g for ocular tissues and ng-eq/mL for tears and aqueous humor).

Pharmacokinetics of cyclosporin A after single doses of ^{3}H-CsA 0.2% emulsion

Following topical administration, the ocular absorption and disposition of a single dose of ^{3}H-CsA 0.2% emulsion was investigated in albino rabbits and in beagle dogs. The change in concentration of ^{3}H-CsA in various ocular tissues over time is shown in Figure 1 (albino rabbits) and Figure 2 (dogs). In addition, peak concentration values (C_{max}) and AUC_{0-96} for both rabbits and dogs are shown in Table 3. Terminal half-life values for ^{3}H-CsA are shown in Table 4. In both the rabbit and dog, high concentrations of ^{3}H-CsA were measured in the tears and extraocular tissues soon after dose instillation (20 minutes and 60 minutes, respectively). Also in both species, ^{3}H-CsA was retained by the extraocular tissues for extended periods of time but there was very little systemic exposure.

Rabbits

In the rabbit, high levels of ^{3}H-CsA were measured in the ocular tissues that were in immediate contact with the dosing solution, namely the conjunctiva and nictitating membrane. After 20 minutes, the concentration of ^{3}H-CsA was 1080 ng-eq/g in the cornea, and 836 ng-eq/g and 753 ng-eq/g in the upper and lower conjunctiva, respectively (Fig. 1).

Peak concentrations of ^{3}H-CsA were measured in the upper and lower conjunctiva after 1 hour (955 and 773 ng-eq/g, respectively), but the concentration of ^{3}H-CsA in the cornea continued to rise for several hours, reaching a peak at 12 hours postdose (1320 ± 220 ng-eq/g). During the first 3 hours after dosing, the concentration of ^{3}H-CsA in the sclera (range 154 to 272 ng eq/g) was lower than in the other ex-

Table 2. Recovery of radioactivity from rabbit ocular tissues following topical administration of ^{3}H-cyclosporin A[a] to the left eye

Tissue	Time (hr)[b]	Total tissue radioactivity (DPM)	Extracted radioactivity (DPM)	Extraction efficiency (%)
Tear	2	119000	11800	99.2
	6	30000	29800	99.3
	24	14900	14500	97.3
Conjunctiva	2	61400	618000	99.3
	6	13800	13700	99.3
	24	3760	3730	99.2
Cornea	2	65100	64800	99.5
	6	31400	31300	99.7
	24	34400	34200	99.4
Sclera	2	11800	11700	99.2
	6	9140	9060	99.1
	24	3080	3060	99.4
Iris-Ciliary Body	2	1490	1490	100.0
	6	772	772	100.0
	24	1230	1230	100.0
Choroid-Retina	2	8200	8120	99.9
	6	2570	240	98.8
	24	1220	1220	100.0
Lacrimal Gland	2	2720	2580	93.8
	6	2020	1870	92.6
	24	1350	1270	94.1

[a] rabbits received a single 50 µl dose of a 0.2% emulsion of ^{3}H-CsA; [b] n = 3 at each time point

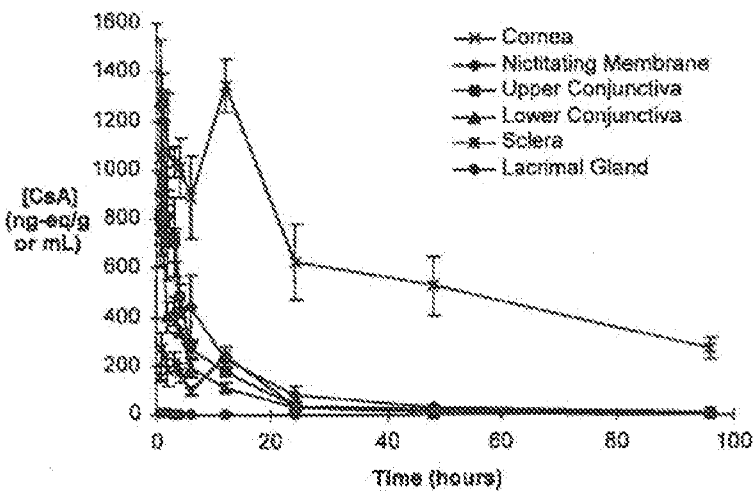

Figure 1. Changes in ^{3}H-cyclosporin A concentration with time after administration of a single topical dose to the left eyes of albino rabbits in a 0.2% emulsion. The error bars represent standard errors.

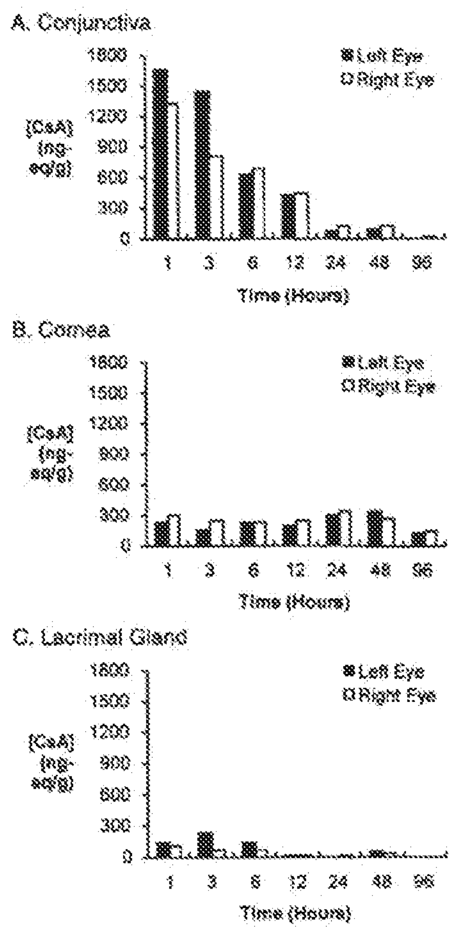

Figure 2. Changes in ^{3}H-cyclosporin A concentration with time after administration of a single topical dose to both eyes of beagle dogs in a 0.2% emulsion. Amount of ^{3}H-cyclosporin A in (a) conjunctiva; (b) cornea, and (c) lacrimal gland.

Table 3. C_{max} and AUC values for ^{3}H-cyclosporin A after instillation of a single dose of 0.2% emulsion to albino rabbits and beagle dogs

	C_{max} (ng-eq/g)			$AUC_{0\text{-}96hr}$ (ng-eq.h/g)		
	Albino rabbit[b]		Beagle dog[c]	Albino rabbit[b]		Beagle dog[c]
Tissue	Treated eye	Untreated eye	Treated eye	Treated eye	Untreated eye	Treated eye
Tears[a]	79000	47.3	27640	409000	1990	430068
Cornea	1340	4.03	311	57500	38.6	23516
Upper conjunctiva	955	6.88	1490	7270	17.4	17824
Lower conjunctiva	773	7.23	ND	5180	25.7	ND
Sclera	272	3.00	94.6	5180	13.4	1303
Aqueous humor[a]	13.7	0.591	0.150	88.4	48.1	8.7
Iris-ciliary body	63.5	3.13	11.2	1670	13.8	605
Lens	3.01	0.336	1.55	146	4.06	73.3
Vitreous humor[a]	0.895	0.141	0.250	22.4	6.13	0.600
Choroid-retina	89.8	13.4	6.95	1550	584	214
Optic nerve	4.90	4.27	9.98	59.8	NC	243
Nictitating membrane / nictitans gland	1540	2.38	205	10400	36.5	6080
Lacrimal gland	8.90	1.97	148	181	51.9	2893
Blood[a]	0.745	0.745	0.520	26.8	26.8	10.1
Plasma[a]	0.652	0.652	0.090	44.3	44.3	0.100

[a] units refer to mL fluid; [b] value for left eye only; [c] mean values for both eyes. ND = not determined

traocular tissues. Furthermore, the C_{max} in the sclera was lower than the C_{max} in the nictitating membrane (272 ng-eq/g compared to 1540 ng-eq/g).

With the exception of the cornea, the level of ^{3}H-CsA in extraocular rabbit tissues remained relatively constant for the first 3 hours and, thereafter, dropped slowly. In the cornea, significant levels of ^{3}H-CsA could still be measured 96 hours after dose instillation (273 ng-eq/g), but by this time the concentration of ^{3}H-CsA in the conjunctiva, nictitating membrane, and sclera had dropped to approximately 4 to 7 ng-eq/g.

In general, ocular tissue C_{max} values for ^{3}H-CsA were measured 20 minutes to 1 hour after dose instillation showing that ^{3}H-CsA was rapidly absorbed into the eye (Table 3). For example, C_{max} was measured in the lacrimal gland at 1 hr post-dose (8.90 ± 4.63 ng-eq/g). However, the intraocular tissue C_{max} values were significantly lower than the extraocular tissue C_{max} values; showing that ^{3}H-CsA did not significantly penetrate into intraocular tissues.

Ocular tissue distribution was also characterized by $AUC_{0\text{-}96hr}$. Highest values were measured in the tears, nictitating membrane, and cornea; the lowest were measured in the optic nerve and vitreous humor (Table 3).

The terminal elimination half-life ($T_{1/2}$) of ^{3}H-CsA in most rabbit ocular tissues was relatively long and ranged from 16 hours (nictitating membrane), to 44 hours (iris-ciliary body) (Table 4).

Most of the tissues of the untreated eye contained detectable but relatively low levels of radioactivity during the first 48 hours after dosing (Table 3). In all tissues measured, the level of radioactivity was lower in the untreated eye than the treated eye. In most tissues (tears, nictitating membrane, upper and lower conjunctiva, cornea, sclera, aqueous humor, and iris-ciliary body) the radioactivity detected in the untreated eye was less than 5% of that detected in the treated eye. However, the levels for the untreated eye were higher in the lens (11% of the level in the treated eye), choroid-retina (15%), vitreous humor (16%), lacrimal gland (22%), and optic nerve (87%).

Beagle dogs

Following topical administration of a single dose of ^{3}H-CsA 0.2% emulsion to both eyes of beagle dogs, ^{3}H-CsA was distributed in a pattern similar to that described for rabbits. The highest concentration of ^{3}H-CsA was recorded in tear samples recovered 6 hours after dose administration (mean 27640 ng-eq/mL); by 96 hours these levels had dropped significantly, (184 ng eq/g) (Fig. 2). Peak extraocular tissue concentrations of ^{3}H-CsA were recorded in the conjunctiva 1 hour postdose (1494 ng-eq/g), in the cornea 24 hours postdose (311 ng-eq/g), and in the sclera 1 hour postdose (95 ng-eq/g) (Fig. 2). Also consistent with results in the rabbit, by 96 hours postdose the extraocular level of ^{3}H-CsA was very low in all tissues except the cornea.

Consistent with results from the rabbit, C_{max} values were significantly greater in the extraocular compared to intraocular tissues (Table 3). The highest concentration of ^{3}H-CsA was recorded in conjunctiva, nictitans gland, and the lacrimal gland of the dog. The highest intraocular tissue levels of ^{3}H-CsA were measured in the iris/ciliary body at 48 hours postdose (C_{max} = 11.2 ng-eq/g).

Ocular tissue distribution was also characterized by $AUC_{0\text{-}96hr}$. Highest values were measured in the tears, cornea, and conjunctiva; the lowest were measured in the lens,

Table 4. Terminal half-life of ^{3}H-cyclosporin A after instillation of a single dose of 0.2% emulsion to albino rabbits and beagle dogs

	Terminal $T_{1/2}$ (hr)	
Tissue	Albino rabbit[b]	Beagle dog[c]
Tears[a]	17.0	16.1
Cornea	42.0	NC
Upper conjunctiva	27.0	16.7
Lower conjunctiva	26.0	NA
Sclera	30.0	26.1
Aqueous humor[a]	NC	NC
Iris-ciliary body	44.0	NC
Lens	NC	NC
Vitreous humor[a]	NC	NC
Choroid-retina	43.0	42.1
Orbital fat	ND	42.2
Optic nerve	NC	31.8
Nictitating membrane/ nictitans gland	16.0	29.5
Lacrimal gland	31.0	14.6

[a] units refer to mL fluid; [b] value for left eye only; [c] mean values for both eyes; NA = not applicable; NC = not calculable; ND = not determined

vitreous humor, and aqueous humor (Table 3).

Topically administered ^{3}H-CsA emulsion also had a long-terminal half-life in dog ocular tissue; however, interanimal variation and an apparently long elimination phase meant that mean $T_{1/2}$ values could not be calculated for all tissues, including the cornea. Calculated values ranged from 14.6 hours (lacrimal gland) to 42.5 hours (orbital fat) (Table 4).

Effect of increasing ^{3}H-CsA concentration on penetration into rabbit ocular tissue after single doses of ^{3}H-CsA emulsion

In order to show the relationship between the concentration of ^{3}H-CsA in various ocular tissues and increasing dose, female albino rabbits were treated with either ^{3}H-CsA 0.05%, 0.2%, or 0.4% emulsion. Figure 3 illustrates the change in concentrations of ^{3}H-CsA with time in the target tissues of the cornea, lacrimal gland and conjunctiva. After administration of all three test doses, the highest concentrations of ^{3}H-CsA were measured in cornea and conjunctiva. As previously seen in the single dose experiments, the concentration of ^{3}H-CsA in intraocular tissues was significantly lower, after

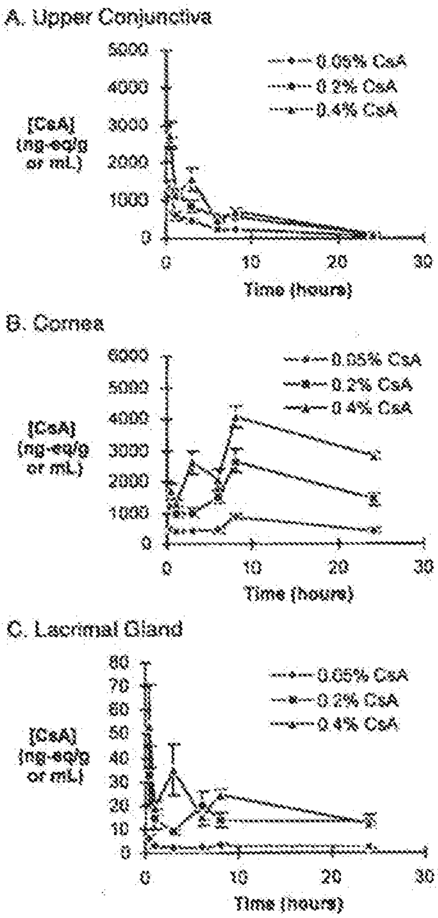

Figure 3. Changes in ^{3}H-cyclosporin A concentration with time after administration of a single topical dose to the left eyes of albino rabbits in a 0.05%, 0.2% or 0.4% emulsion. Amount of ^{3}H-cyclosporin A in (a) upper conjunctiva; (b) cornea, and (c) lacrimal gland. The error bars represent standard deviations.

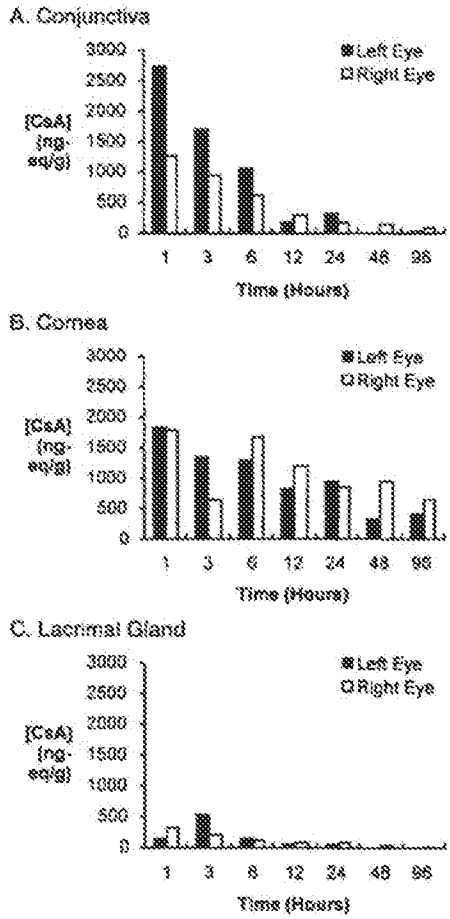

Figure 4. Changes in ^{3}H-cyclosporin A concentration with time after administration of multiple topical doses to both eyes of beagle dogs in a 0.2% emulsion. Amount of ^{3}H-cyclosporin A in (a) upper conjunctiva; (b) cornea, and (c) lacrimal gland.

Table 5. Effect of dose concentration on the penetration of ^{3}H-cyclosporin A emulsion into ocular tissues of albino rabbits following a single dose to both eyes

A. Maximum tissue concentration (C_{max})

Ocular tissue	Tissue C_{max} (ng-eq/g) Dose[a] 0.05%[b]	0.2%[c]	0.4%[d]
upper conjunctiva	1320 ± 500	2610 ± 290	2750 ± 900
lower conjunctiva	946 ± 276	2180 ± 250	2840 ± 1300
cornea	873 ± 232	2650 ± 910	4050 ± 800
aqueous humor	0.838 ± 0.323	1.99 ± 0.41	4.82 ± 0. 85
iris-ciliary body	13.7 ± 4.3	32.9 ± 16.0	85.9 ± 38.8
lacrimal gland	6.16 ± 3.69	32.7 ± 30.0	53.3 ± 43.5

[a] rabbits received a single 50 µl dose of the emulsion of ^{3}H-cyclosporin A in each eye;
[b,c,d] Values shown are mean ± SD for 5 to 6 animals.

B. Area under the tissue concentration/time curve (AUC)

Ocular tissue	AUC_{0-24hr} (ng-eq.hr/g) Dose[a] 0.05%[b]	0.2%[c]	0.4%[d]
upper conjunctiva	5560 ± 240	12400 ± 600	15000 ± 1400
lower conjunctiva	4690 ± 240	9730 ± 670	14900 ± 1600
cornea	14100 ± 1000	43300 ± 3800	73300 ± 3300
aqueous humor	12.5 ± 1.3	30.4 ± 4.4	69.4 ± 8.4
iris-ciliary body	246 ± 21	675 ± 99	1220 ± 80
lacrimal gland	64.4 ± 4.3	332 ± 39	493 ± 41

[a] rabbits received a single 50 µl dose of the emulsion of ^{3}H-cyclosporin A in each eye;
[b,c,d] Values shown are mean ± SD for 5 to 6 animals.

all three doses, than in the extraocular tissues (Table 5A).

Peak concentrations of ^{3}H-CsA were detected in the conjunctiva and lacrimal gland after only 20 minutes. In the cornea, the concentration of ^{3}H-CsA continued to rise for several hours reaching a peak at 8 hours postdose and then dropped very slowly; concentrations of 422, 1410, and 2810 ng-eq/g were measured 24 hours after dosing with the 0.05%, 0.2%, and 0.4% emulsions, respectively (Fig. 3B).

After administration of the 0.05% and 0.2% doses only, the AUC_{0-96hr} value measured in the upper conjunctiva was higher than that in the lower conjunctiva (Table 5B). Increasing dose of cyclosporin A by 4-fold (from 0.05% to 0.2%) resulted in a 2.2-fold, 3.1-fold, and 5.2-fold increase in the AUC_{0-96hr} value for the upper conjunctiva, cornea, and lacrimal gland respectively ($P < .001$ for all three tissues). Doubling the dose (from 0.2% to 0.4%) resulted in a 1.2-fold, 1.7-fold, and 1.5-fold increase for the same three tissues ($P = .01$; $P < .001$, and $P < .01$, respectively).

Although there was a consistent increase in the ocular concentration of ^{3}H-CsA after instillation of increasing concentrations, levels of ^{3}H-CsA in the conjunctiva and cornea actually showed a less than proportional increase with dose, while the concentration of ^{3}H-CsA increased proportionately with dose in the lacrimal gland and all intraocular tissues (Table 5A).

Pharmacokinetics of cyclosporin A after multiple doses of ^{3}H-CsA 0.2% emulsion

The ocular absorption and disposition of ^{3}H-CsA after administration of multiple doses was studied in Beagle dogs given ^{3}H-CsA 0.2% emulsion twice daily for 7 days to both eyes.

The concentration of ^{3}H-CsA in tears was at least 10-fold higher than in other ocular tissues and maximal in tear samples recovered 3 hours after the final dose (288730 ng-eq/g). Concentrations in surface ocular tissues were maximal 1 hour after dose administration (Fig. 4). As was seen after administration of single doses, there was very limited penetration of ^{3}H-CsA across the cornea after administration of multiple doses. Extraocular tissues (conjunctiva, cornea, and sclera) contained much higher levels of ^{3}H-CsA than did the intraocular tissues (Table 6). The mean concentration of ^{3}H-CsA in the conjunctiva and cornea was 2007 ng-eq/g and 1809 ng-eq/g, respectively. The mean concentration of ^{3}H-CsA in the sclera (125 ng-eq/g) was much lower than in the conjunctiva and cornea, but was also significantly higher than in intraocular tissues. The mean blood level following multiple doses was not much higher than that following single doses (Tables 3 and 6).

To estimate the extent of tissue accumulation, the ratio of the C_{max} value after multiple doses (C_{max-MD}) to C_{max} after

Table 6. Ocular cyclosporine concentrations in beagle dogs following multiple doses of 0.2% ^{3}H-cyclosporin A emulsion

Ocular tissue	Tissue C_{max} after multiple dosing (ng-eq/g)	Ratio $C_{max\text{-}MD}$: $C_{max\text{-}SD}$
cornea	1809	5.82
conjunctiva	2007	1.35
sclera	125	1.32
aqueous humor	0.630	4.20
iris-ciliary body	35.7	3.19
lens	3.04	1.96
vitreous humor	0.280	1.10
choroid-retina	12.3	1.77
optic nerve	12.5	1.25
nictitans gland	690	ND
lacrimal gland	357	2.41
blood	1.15	ND
plasma	0.330	ND

MD = multiple doses (one drop b.i.d for 7 days); SD = single dose. Ratio of $C_{max\text{-}SD}$:$C_{max\text{-}SD}$ were calculated using C_{max} for single dose values from Table 3. ND = not determined

single doses ($C_{max\text{-}SD}$) was determined for extraocular and intraocular tissues (Table 6). These ratios reflect the variable accumulation of ^{3}H-CsA in different ocular tissues during the 7 days of b.i.d. dosing. For example, only limited accumulation of ^{3}H-CsA took place in both the conjunctiva and sclera (ratios of 1.35 and 1.32, respectively), but there was significant accumulation of ^{3}H-CsA in the cornea (ratio of 5.82), lens, lacrimal gland, and iris-ciliary body (ratio of 3.19).

Very low intraocular tissue concentrations of ^{3}H-CsA were measured after multiple as well as single dosing. Although there was some accumulation of ^{3}H-CsA in intraocular tissues during the 7-day dosing period (as was observed in extraocular tissues), intraocular accumulation was not as extensive (Table 6).

As was seen in the single-dose study in the dog, ^{3}H-CsA had an apparently long elimination phase in most ocular tissues after multiple dosing, which, combined with the significant interanimal variability, meant that mean $T_{1/2}$ values could be calculated for only the conjunctiva (34.1 hr), nictitans gland (17.7 hr), and lacrimal gland (28.0 hr). Although the mean $T_{1/2}$ value could not be calculated for the cornea, significant levels of ^{3}H-CsA were measured in the cornea 96 hours after the last dose, consistent with the cornea acting as a reservoir for ^{3}H-CsA.

Pharmacokinetics of cyclosporin A after single and multiple dosing in an ointment formulation

The pharmacokinetics of ^{3}H-CsA in rabbits were examined after single and multiple dosing in the corn oil ointment to both eyes of albino rabbits. In the single dose study, the ocular tissues were examined at selected time points between 0 to 96 hr after topical administration of 20 µL of ointment. For the multiple dose study, each rabbit received ointment daily for 7 days. Values for C_{max} in ocular tissues after single and multiple dosing are compared in Table 7.

When administered in the corn oil ointment, ^{3}H-CsA was rapidly absorbed into the eye; significant levels were observed in all ocular tissues 20 minutes after the administration of a single dose. The highest concentration of ^{3}H-CsA detected was in the cornea and this was consistently 100 to 1,000 times that found in the blood. The mean C_{max} in the cornea was observed after 5 hours. The elimination $T_{1/2}$ was approximately 40 hours. As with the emulsion formulation, significant levels (approximately 140 ng-eq/g) of ^{3}H-CsA were still detected in the cornea after 96 hours. Peak concentrations in most other tissues (both extraocular and intraocular) were observed within the first hour after dosing.

Table 7. Ocular cyclosporine concentrations in albino rabbits following single and multiple doses of an 0.2% ^{3}H-cyclosporin A ointment to both eyes

Ocular tissue	Single dose Tissue C_{max} (ng-eq/g)[a]	Multiple doses[c] Tissue C_{max} (ng-eq/g)[b]	Ratio $C_{max\text{-}MD}$: $C_{max\text{-}SD}$
cornea	1548 ± 548	6011 ± 6800	3.9
conjunctiva	ND	ND	NA
sclera	17.10 ± 4.83	35.17 ± 10.01	2.1
aqueous humor	9.590 ± 6.01	19.26 ± 6.72	2.0
iris-ciliary body	35.03 ± 16.81	108.7 ± 24.55	3.1
lens	3.890 ± 1.71	39.56 ± 5.51	10.1
nictitans gland	ND	ND	NA
vitreous humor	0.900 ± 0.33	0.8100 ± 0.31	0.9
choroid-retina	2.910 ± 0.94	4.620 ± 2.31	1.6
optic nerve	ND	ND	NA
lacrimal gland	11.96 ± 14.62	110.1 ± 230.6	9.2

[a,b] n =3 at each time point; [c] Multiple dose = 20 µL applied b.i.d. for 7 days. MD = multiple doses; SD = single dose; ND = not determined

Low and highly variable concentrations of radioactivity were measured in the blood following both single and multiple applications; the mean level was 49.8 ± 58.0 after a single application and 7.44 ± 4.42 after multiple applications. The high mean blood level and large standard deviation following single applications was likely due to experimental error resulting in ingestion of ointment during grooming. This contention is supported by the fact that very low blood levels were seen in all other experiments in which they were measured (see Tables 3 and 6).

During multiple dosing, ^{3}H-CsA accumulated in the ocular tissue. The ratio of C_{max}[multiple]:C_{max}[single] was greatest in the lens and lacrimal gland (ratios of 10.1 and 9.2, respectively) whereas there was very little accumulation in either the vitreous humor or choroid-retina (ratios 0.9 and 1.6, respectively) (Table 7).

The terminal $T_{1/2}$ of ^{3}H-CsA after multiple dosing was slightly prolonged in tissues with high accumulation of the drug. In tissues such as the lens and vitreous humor, that eliminate drugs slowly, a slow prolonged absorption lead to continually increasing concentrations over the 96-hour period postdose.

Ocular retention time of cyclosporine emulsion and emulsion vehicle

Interferometric studies were carried out to evaluate the ocular retention time (ORT) of the cyclosporin A emulsion formulation and its vehicle. Since the amount of oil in the emulsion formulation increases with the concentration of cyclosporin A, the vehicles for several different cyclosporin A concentrations were tested. Test substances were instilled into the left eyes of beagle dogs, while the right eyes were left untreated to serve as controls.

A baseline examination of the tear film was carried out prior to instillation of test substances. Mean ORT after instillation increased in parallel with increasing concentration of cyclosporin A in both the cyclosporin A-containing emulsions and their corresponding vehicles. Overall, only a moderate increase in ORT with concentration was observed in the vehicle emulsions (Fig. 5a). The vehicle for cyclosporin A 0.01% had an ORT of 1.5 hours, while the vehicle for cyclosporin A 0.2% had an ORT of 2.75 hours. A similar increase in ORT with increasing concentration was seen for the cyclosporin A emulsions (Fig. 5b); cyclosporin A 0.05% had an ORT of 1.75 hours, cyclosporin A 0.2% had an ORT of 3.1 hours, and cyclosporin A 0.4% had an ORT of 4.2 hours.

Discussion

The most important finding of the present report was that topically applied cyclosporin A was rapidly absorbed into the ocular tissues known to be affected in dry eye disease (the conjunctiva, cornea, and lacrimal gland) at concentrations sufficiently high to suppress inflammatory processes. The extraocular tissues retained high concentrations of cyclosporin A for an extended period of time after a single dose – from 3 hours (lacrimal gland) to 96 hours (cornea); the result of a long elimination $T_{1/2}$ in these tissues. This suggests that the extraocular tissues – in particular the cornea – may act as a reservoir distributing cyclosporin A to the rest of the eye. Much lower concentrations of cyclosporin A were found in intraocular tissues and there was minimal drug absorption into the systemic circulation.

These findings are in good agreement with the results of earlier studies by Weiderholt *et al.* (17) and Kaswan (16). As in the present study, Wiederholt *et al.* (17) found that cyclosporin A was not metabolized by ocular tissues and that the ocular radioactivity concentrations following administration of radiolabeled drug could therefore be considered equivalent to cyclosporine A concentrations. Forty-eight hours after topical administration of a single dose of 2% cyclosporin A in castor oil to rabbits, Weiderholt *et al.* found concentrations of cyclosporin A in the cornea to be high and persistent, and concluded that this tissue served as a reservoir of cyclosporin A. They suggested that the slow release of cyclosporin A from the corneal epithelium was responsible for the long retention time of cyclosporin A and that the corneal stroma was acting as a barrier limiting the penetration of cyclosporin A into intraocular tissues. Kaswan (16) studied the penetration of topically administered cyclosporin A in albino rabbits after six applications of 7 µL of 1% cyclosporin A in an olive oil formulation and found that, within 1 hour of dosing, extraocular tissue levels of cyclosporin A had increased to > 50 ng/g and that these concentrations were maintained in the cornea and sclera for 24 hours after dosing. However, in both the Kaswan study and the present report, the concentrations of cyclosporin A in the aqueous humor and vitreous humor remained below 50 ng/g.

The results from the present report suggest that the cyclosporin A concentrations in the extraocular tissues after topical administration were well within the range required for effective modulation of the immune response. Although it is generally agreed that a blood level of between 200 to 800 ng/mL should be obtained in patients undergoing internal organ transplantation (25), significantly lower levels of cyclosporin A (50 to 300 ng/g) in extraocular tissues are expected to be sufficient to adequately modulate the local immune response after topical administration to the eye (9, 16).

In all of the studies in the present report, blood cyclosporin A concentration was minimal. In order to maximize the potential for systemic exposure, a particularly large drop size (50 µL rather than the more typical 35 µL) was used in the experiments on albino rabbits, and the systemic exposure was still less than 0.75 ng-eq/g (C_{max}). As adverse effects associated with systemic cyclosporin A appear to be dose dependant (26), the low levels observed in the present studies suggest that the clinical use of topical cyclosporin A ophthalmic castor oil-in-water emulsions or corn oil ointments is unlikely to lead to any of the adverse effects associated with systemic administration of cyclosporin A.

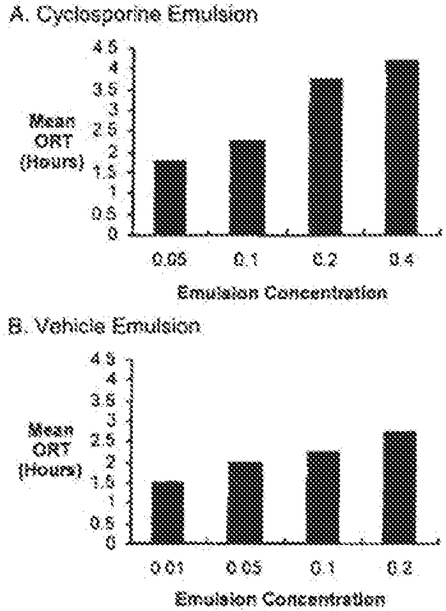

Figure 5. Ocular retention time of the (a) cyclosporin A emulsion and (b) vehicle emulsion in the left eyes of beagle dogs. Each bar represents the average of two separate experiments.

Most topical ocular drugs reach intraocular tissues by diffusion across the the corneal and conjunctival epithelium and by systemic contribution (27). The presence of detectable levels of radioactivity in the tissues of untreated eyes following single doses in rabbits suggests that systemically absorbed cyclosporin A may be contributing to the ocular distribution of the drug to certain tissues within the treated eye. In most tissues of the untreated eye, the radioactivity measured was less than 5% of that found in the treated eye. However, the radioactivity measured in the lens, choroid-retina, vitreous humor, lacrimal gland, and optic nerve of the untreated eye ranged from 11% to 87% of that found in the treated eye. Of particular relevance to the use of topical cyclosporin A in the treatment of dry eye disease was the fact that radioactivity in the lacrimal gland of the untreated eye was 22% of that in the treated eye. This suggests that topically applied cyclosporin A reaches this critical tissue both by diffusion through nearby tissues and by reabsorption from the blood.

Vernillet and colleagues (1991) (28) also found measurable levels of cyclosporin A in the untreated eye following unilateral dosing in rabbits. These investigators used a much higher concentration and much more frequent dosing of cyclosporin A (a 1% solution administered 5 times daily) than was used in the present studies, and a less sensitive radioimmunoassay to detect drug levels in tissue and blood. They found comparable levels of cyclosporin A in the tissues of both the treated and untreated eyes, but were unable to detect any cyclosporin A in the blood. They speculated that "[Cyclosporin A] could be diffused from the site of administration to the optic chiasma and/or to a local vascular system irrigating the lacrimal glands and the ocular globe, and then be distributed into the fellow eye." Although this explanation cannot be definitively refuted or validated by the results of the present study, the finding of low but detectable levels of cyclosporin A in the blood suggests that absorption of blood-born drug may be one way in which cyclosporin A reaches the tissues of the untreated eye.

The long ocular retention time of the emulsion vehicle demonstrated in the present report may have contributed to the prolonged absorption of cyclosporin A into extraocular tissues by increasing the contact time between the drug formulation and the surface of the eye. However, this long ocular retention time may also provide additional benefits to patients with dry eye disease. The castor oil droplets in the emulsion may form a lipid layer over the tear film, possibly reducing the evaporation of the limited natural tears produced by these patients while the emulsion remains in the eye.

The results presented here show that the absorption and distribution of cyclosporin A was not significantly influenced by the presence or absence of iris pigment. There was no difference in the accumulation of cyclosporin A by the pigmented ocular tissues of the dog (such as the iris-ciliary body and choroid-retina) as compared with the corresponding non-pigmented tissues of the rabbit. This suggests that cyclosporin A does not bind avidly to melanin.

For the most part, the relative accumulation of cyclosporin A in various ocular tissues was similar in rabbits and dogs. When the tissues were ranked according to either C_{max} or AUC, the same tissue from the two different species tended to be within 1 or 2 rank levels of each other. One notable exception was the lacrimal gland; it contained the fifth highest concentration in the dog and only the ninth or tenth highest concentration in the rabbit. This difference is undoubtedly due to anatomical differences between the two species; the gland in the rabbit is larger than in the dog and the drug would be distributed through a larger tissue volume, and the gland in the rabbit lies deeper within the orbit than does the gland in the dog (29, 30).

In the present studies, the ointment and emulsion formulations were associated with similar cyclosporin A pharmacokinetics. This suggests that it would be appropriate to differentiate between these two formulations on the basis of their clinical characteristics. For example, cyclosporin A ointment has been associated with early burning, redness, and itching after topical administration to humans (13), while it is possible that the emulsion formulation will be better tolerated.

Extrapolating the pharmacokinetic data provided here for the rabbit and dog to human patients is somewhat problematic considering the interspecies differences in blink frequency, corneal permeability, and aqueous humor dynamics between animals and humans. However, a mean cyclosporin A concentration of 236 ± 42 ng/g was reported for explanted human corneas (obtained from patients receiving corneal transplants) following topical dosing with 2% cyclosporin in an olive oil formulation (10). This suggests that the results for animals and humans may be similar and that topical administration of cyclosporin A to patients with dry eye disease

should provide levels of cyclosporin A in extraocular tissues adequate to modulate the local immune response without significant systemic exposure.

In conclusion, the pharmacokinetic properties described here suggest that after topical administration in a castor oil-in-water emulsion formulation, cyclosporin A is rapidly absorbed by extraocular tissues; penetrating into the cornea, conjunctiva, and lacrimal gland in amounts sufficient to provide immunomodulation. Furthermore, the long retention time of cyclosporin A suggests that the local immune response could be modulated for extended periods and successfully control the ocular inflammatory processes characteristic of dry eye disease.

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A Randomized Trial of Topical Cyclosporin 0.05% in Topical Steroid–Resistant Atopic Keratoconjunctivitis

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Objective: To evaluate the short-term efficacy and safety of topical cyclosporin A 0.05% in the treatment of patients with severe, steroid-resistant atopic keratoconjunctivitis (AKC).

Design: Multicenter, placebo-controlled, double-masked, randomized trial.

Participants: Twenty-two patients with AKC refractory to topical steroid treatment.

Intervention: Patients were randomly assigned (1:1) to treatment with topical 0.05% cyclosporin A eyedrops or a placebo (artificial tears) for a period of 28 days, 6 times daily during the first 2 weeks and 4 times daily during the last 2 weeks.

Main Outcome Measures: Symptoms (itching, tearing, discomfort, mucous discharge, and photophobia) and signs (bulbar conjunctival hyperemia, upper tarsal conjunctival papillae, punctate keratitis, corneal neovascularization, cicatrizing conjunctivitis, and blepharitis) of AKC recorded on the day of enrollment and at the end of the treatment period.

Results: A composite score computed by summing the severity grade over all 5 symptoms and 6 signs of AKC for each patient indicated a greater improvement in the cyclosporin A group relative to the placebo at the end of the 4-week treatment period ($P = 0.048$ and $P = 0.002$, for symptoms and signs, respectively). No adverse effects of the treatment with cyclosporin A 0.05% eyedrops were observed.

Conclusions: Topical cyclosporin A 0.05% seems to be safe and have some effect in alleviating signs and symptoms of severe AKC refractory to topical steroid treatment. *Ophthalmology 2004;111:476–482 © 2004 by the American Academy of Ophthalmology.*

Atopic keratoconjunctivitis (AKC) is a severe and chronic form of ocular allergy manifesting with multiple ocular surface disorders in the context of atopic dermatitis. Although rare, AKC is the most debilitating of the allergic conjunctival diseases, owing to its chronicity and ability to cause conjunctival scarring and loss of vision due to frequent corneal complications.

The pathogenesis of AKC is not fully understood, and the initiating event is unknown. Histopathologic examination of conjunctival biopsies from patients with AKC demonstrates goblet cell proliferation, epithelial pseudotubular formation, and a pronounced invasion of the epithelium by degranulating eosinophils and mast cells.[1] The substantia propria shows a dramatic infiltration with CD25+ T lymphocytes (activated T cells expressing the interleukin-2 receptor), macrophages, and dendritic cells (HLA-DR+, HLA-CD1+). The constellation of these immunopathogenic findings points to a complex immunoregulatory dysfunction at the ocular surface, which is far more involved than a simple type I hypersensitivity reaction. The influx of T helper lymphocytes producing interleukin-2 and interferon-γ suggests that a delayed hypersensitivity reaction predominates,[2] with resultant subepithelial fibrosis and structural alterations of the ocular surface.

Atopic keratoconjunctivitis is a chronic disease that persists for many years, with a high rate of visual impairment. The currently available therapies are aimed at relieving the patient symptoms, at best. No evidence exists showing a significant effect on the disease course. Corticosteroids are the mainstay of treatment, with dramatic improvement in acute symptoms. However, the efficacy of steroids in preventing the crippling consequences of AKC is not known. In the largest case series from a tertiary ophthalmic center, significant keratopathy developed in approximately two

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thirds of patients managed with the usual oral antihistamine, topical mast cell stabilizer, and intermittent topical steroid regimen.[3,4] Furthermore, risks of glaucoma, superinfection with viruses and bacteria due to local immunosuppression, delayed wound healing, and cataract induction warrant cautious use of topical steroids. Therefore, the need to address the complex, chronic, and multifactorial nature of the disease continues.

Cyclosporin A is an immunomodulator that specifically inhibits CD4+ T lymphocyte proliferation via inhibition of interleukin-2 receptor expression.[5] Cyclosporin A also has direct inhibitory effects on eosinophil and mast cell activation and release of mediators, which seem to be important in its role in the treatment of allergic inflammation.[6] In fact, topical cyclosporin A 2% in maize oil has been used previously in the treatment of patients with topical steroid-dependent AKC, and demonstrated a significant steroid-sparing effect.[7] However, various dose-limiting side effects related to both the vehicle and the medication itself were encountered.

In a randomized, placebo-controlled, double-masked, multicenter trial, we investigated the short-term efficacy of topical cyclosporin A 0.05% in patients with severe AKC refractory to topical steroid treatment.

Materials and Methods

Patients

This study was conducted at 3 centers: The Wilmer Eye Institute, Baltimore, Maryland (14 patients); Moorfields Eye Hospital, London, United Kingdom (6 patients); and Bascom Palmer Eye Institute, Miami, Florida (2 patients). The study protocol was approved by the institutional review board at each site. Adult patients with a diagnosis of AKC who were willing to comply with the protocol and who provided informed consent were enrolled in the study. All patients were known to have had a long-standing AKC, diagnosed based on the criteria outlined by Hogan[8]: briefly, a chronic conjunctivitis and progressive keratitis in association with atopic dermatitis, and presence of a hereditary allergic tendency manifesting as hay fever, rhinitis, asthma, and urticaria. All patients had been treated with a variety of topical medications before enrollment. At the time of enrollment, all but one patient (see "Results" for details) had been using topical steroids for a period of at least 2 weeks, and all remained refractory with persistent/progressive inflammation (presence of at least one moderate to severe sign and/or symptom; see below). Patients with additional causes of ocular inflammation and those with systemic diseases other than atopic dermatitis who were taking systemic corticosteroids, antihistamines, nonsteroidal anti-inflammatory drugs, or immunosuppressives were excluded. Contact lens wearers were also excluded.

Treatment

Patients were assigned, based on a predetermined randomization list generated by computer, to receive either topical cyclosporin A 0.05% (Restasis, Allergan Inc., Irvine, CA) or preservative-free artificial tears (Refresh Tears, Allergan). Allocation coding was undisclosed until all patients had completed the study. Both patients and examining physicians were masked to the identity of the drops used and remained so for the duration of the trial. Drops were in identical single-use droppers, labeled in a masked fashion. The treatment lasted 4 weeks, during which patients were instructed to administer 1 drop of the test medication in the lower conjunctival cul-de-sac of both eyes, 6 times a day during the first 2 weeks and 4 times a day in the last 2 weeks. While taking the test medication, patients stayed on their pre-enrollment topical therapy (corticosteroid or corticosteroid plus mast cell stabilizer) between day 0 and day 28. During days 29 to 35, no test medication was administered, but pre-enrollment medications continued.

Patient Monitoring

At the enrollment visit, day 0, all patients had anterior segment photographs and received a complete ophthalmic examination, including measurement of visual acuity and intraocular pressure, slit-lamp examination, and dilated fundus examination. All patients were dispensed the test medications, and the treatment was started.

At the study follow-up visits (scheduled on days 7, 14, and 28 after enrollment), a brief ophthalmic examination was performed, consisting of a vision test and slit-lamp examination. When a patient could not make the follow-up visits at day 7 or 14, contact by telephone was made, and the questionnaire was completed by the interviewing physician. For one patient (assigned to cyclosporin A) who missed the day 28 visit, data from the visit on day 21 were substituted for analysis purposes.

At each visit, patients graded the symptoms using the study questionnaire. A slit-lamp examination was performed, and a single masked examiner at each center graded the signs noted at the examination on a 4-point scale. Symptoms included itching, tearing, discomfort, mucous discharge, and photophobia. Signs included bulbar conjunctival hyperemia, upper tarsal conjunctival papillae, punctate keratitis, corneal neovascularization, cicatrizing conjunctivitis, and blepharitis. The questionnaire, summarized in Table 1, was prepared by modifying previously described methods.[7,9]

Patients were also asked to assess their ability to tolerate the test drops and report any side effects experienced. On day 35, 1 week after the discontinuation of the test medication, a final complete ophthalmic evaluation was performed, and slit-lamp pictures were obtained.

Each patient was dispensed the exact number of droppers needed for the duration of the trial. To assess compliance, the patients were asked to return the empty droppers to the investigators at the end of the study. To ensure compliance with the pre-enrollment medications, the patients were reminded to stay on them at each visit.

Statistical Analysis

We used the Wilcoxon 2-sample test to compare the severity of individual symptoms and signs at baseline in the treated and placebo groups, and to compare the change in severity of symptoms and signs from baseline to the 4-week follow-up visit. We also defined a composite score separately for symptoms and signs. This score was computed by summing the severity score over all 5 symptoms (composite range = 0–15, using a 4-point scale for each symptom) and 6 signs (composite range = 0–18, using a 4-point scale for each sign). Individuals, rather than eyes, were the unit of analysis because eyes were not examined independently, and the manifestation of disease and response to treatment tended to be correlated. Persons were classified according to the status of the worse eye, and the worse eye was defined separately for symptoms and signs based on the composite score for each. To partially adjust for multiple comparisons, the P value for statistical significance was set at <0.01.

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Table 1. Grading of Symptoms and Signs

	0	1	2	3
Symptoms				
Itching	No desire to rub or scratch the eye	Occasional desire to rub or scratch	Frequent need to scratch or rub the eye	Constant need to rub or scratch the eye
Tearing	Normal tear production	Positive sensation of fullness of the conjunctival sac without tears spilling over the lid margin	Intermittent, infrequent spilling of tears over the lid margin	Constant, or nearly constant, spilling of tears over the lid margins
Discomfort (including burning, stinging, and foreign body sensations)	Absent	Mild	Moderate	Severe
Discharge	No abnormal discharge	Small amount of mucoid discharge noted in the lower cul-de-sac	Moderate amount of mucoid discharge noted in the lower cul-de-sac and in the marginal tear strip; presence of crust upon awakening	Eyelids tightly matted together upon awakening, requiring warm soaks to pry lids apart; warm soaks necessary to clean eyelids during the day
Photophobia	No difficulty experienced	Mild difficulty with light causing squinting	Moderate difficulty, necessitating dark glasses	Extreme photophobia, causing the patient to stay indoors; cannot stand natural light even with dark glasses
Signs				
Bulbar conjunctival hyperemia	Absent	Mild	Moderate	Severe
Tarsal conjunctival papillary hypertrophy	No evidence of papillary formation	Mild papillary hyperemia	Moderate papillary hypertrophy with edema of the palpebral conjunctiva and hazy view of the deep tarsal vessel	Severe papillary hypertrophy obscuring the visualization of the deep tarsal vessels
Punctate keratitis (superficial epithelial keratitis and punctate staining of the cornea with fluorescein)	No evidence of punctate keratitis	One quadrant of punctate keratitis	Two quadrants of punctate keratitis	Three or more quadrants of punctate keratitis
Neovascularization of cornea (new vessel formation, crossing the limbus onto the clear cornea by ≥2 mm)	No evidence of new vessel formation	Presence of neovascularization in 1 quadrant of cornea	Presence of neovascularization in 2 quadrants of cornea	Presence of neovascularization in ≥3 quadrants of cornea
Cicatrizing conjunctivitis (superficial scarring of the conjunctiva)	No evidence of cicatrization	Presence of subepithelial fibrosis	Presence of fornix foreshortening	Symblepharon formation
Blepharitis (hyperemia and edema of eyelid skin with meibomian gland dysfunction)	No evidence of blepharitis	Presence of mild redness and edema of the eyelid with meibomian gland dysfunction	Moderate inflammation with hyperemia, scales, and scurf of eyelid skin and toothpaste phenomenon	Severe inflammation, with cracks in the eyelid skin, loss of eyelashes, and lid edema

Results

Twenty-two patients met the criteria for inclusion in this clinical trial. The distribution of baseline characteristics in the combined sample, and according to assigned treatment, is shown in Table 2. Overall, 40.9% were female, and the mean patient age was 42.6 years (range, 22–73). There was no statistically significant difference between the 2 groups in terms of gender or mean age. There was no statistically significant difference in the severity of symptoms and signs at the enrollment visit between the 2 groups, although the incidence of cicatrizing conjunctivitis tended to be higher in patients in the placebo group ($P = 0.024$).

Patients were diagnosed with severe AKC and had an average disease duration of 12 years (range, 3 months to 30 years). At the time of enrollment, all patients were still symptomatic (with at least one ≥2 sign and/or symptom), despite treatment with topical steroids for at least 2 weeks (range, 2–7). The average daily treatment was with prednisolone acetate 2 times daily, in both the treated group and the placebo group. Two patients were also taking mast cell stabilizers. Only one patient (who had a history of AKC

Table 2. Distribution of Baseline Characteristics in the Combined Sample and According to Assigned Treatment

Characteristic	Combined Sample (n = 22)	Cyclosporin (n = 10)	Placebo (n = 12)	P
Percent female	40.9	40.0	41.7	0.94
Mean age (SD)	42.6 (14.6)	42.3 (10.6)	42.9 (17.8)	0.92
Disease duration (mos)	132	96	150	0.74
Median score of symptoms	8	8	8	0.94
Median score of signs	10	10	10	0.59

SD = standard deviation.

for 25 years) was not taking topical steroids at the time of enrollment. This patient had failed to respond to a treatment with topical steroids along with oral antihistamines before enrollment in the study. At the time of enrollment, the patient had moderate to severe disease, was getting worse, and was willing to enter the study to try the new medication. Five patients had posterior subcapsular cataracts (22.7%), and 2 had keratoconus (9%). One patient had a history of frequent herpes simplex keratitis (4.5%). Additionally, 2 patients had severe sicca syndrome owing to the ocular surface cicatrization (9%).

Two of the 22 randomized patients contributed no follow-up data. One of these patients was lost to follow-up after the enrollment visit. A second patient opted to withdraw from the study after the first follow-up visit to resume contact lens wear for bilateral keratoconus. Both patients were assigned to the placebo. Thus, the final trial results presented below are based on data for the 20 patients (10 assigned to cyclosporin A treatment, 10 assigned to placebo) who completed the study as scheduled.

The median severity grades for individual symptoms and signs as well as composite scores before and after the treatment period are shown in Table 3. At the end of the 4-week treatment period, patients assigned to cyclosporin A tended to have more improvement in symptoms than did the placebo group (Fig 1). Although the differences for any individual symptom did not attain statistical significance at the $P<0.01$ level, a composite score summing severity grades over all 5 symptoms for each patient did suggest a benefit for treatment with cyclosporin A ($P = 0.048$). With regard to signs of AKC (Fig 2), patients in the treated group, compared with those in the placebo group, showed significantly greater improvement in upper tarsal conjunctival papillae ($P = 0.005$) and punctate keratitis ($P = 0.007$). The treated group also experienced less progression of corneal neovascularization ($P = 0.051$) and greater improvement in blepharitis ($P = 0.055$), although the differences were not statistically significant. The composite score for signs indicated a statistically significant benefit ($P = 0.002$) for treatment with cyclosporin A.

All but one patient tolerated the test medication well. The one patient complained of headaches with use of test medication. This patient was assigned to the placebo group.

The patients who were still symptomatic at the end of the trial were placed on topical cyclosporin A 0.5% (in artificial tears) in addition to the corticosteroid eyedrops they were taking.

Discussion

The results of our trial suggest that topical cyclosporin A is of benefit in the treatment of patients with topical corticosteroid–resistant AKC. Although limited by a small sample size and relatively short duration, these results indicate that improvement in the symptoms and signs of AKC was consistently greater in those assigned to treatment with cyclosporin A 0.05% than in those in the placebo group. Another weakness of the study is the use of artificial tears rather than the actual vehicle, as it is possible that the beneficial effects of the cyclosporin eyedrops could have been due to the vehicle alone.

Table 3. Median Severity Grades for Symptoms and Signs before and after a 4-Week Treatment Period

	Cyclosporin (n = 10)		Placebo (n = 10)		
	Before	*After*	*Before*	*After*	P
Symptoms					
Itching	2.0	1.0	1.5	1.0	0.089
Tearing	2.0	0.5	1.5	1.0	0.20
Discomfort	1.5	0.5	2.0	1.5	0.14
Mucous discharge	1.5	0.5	1.5	1.0	0.13
Photophobia	0.5	0.0	2.0	2.0	0.40
Composite symptom score	8.0	3.0	8.0	8.0	
Median change in composite symptom score	4		0.5		0.048
Signs					
Bulbar conjunctival hyperemia	2.0	1.0	1.5	1.0	0.017
Upper tarsal conjunctival papillae	3.0	1.5	2.0	2.0	0.005
Punctate keratitis	3.0	0.5	1.0	1.5	0.007
Corneal neovascularization	0.0	0.0	0.5	1.0	0.051
Cicatrizing conjunctivitis	0.0	0.0	1.0	1.0	0.99
Blepharitis	2.5	1.0	2.0	2.0	0.055
Composite sign score	10.0	5.0	10.5	9.5	
Mean change in composite sign score	5		−1		0.002

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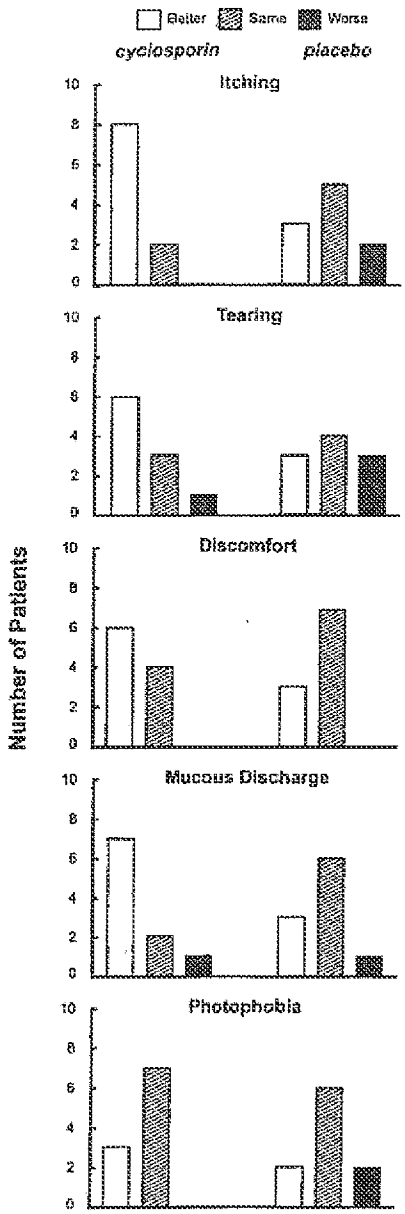

Figure 1. Symptoms of atopic keratoconjunctivitis.

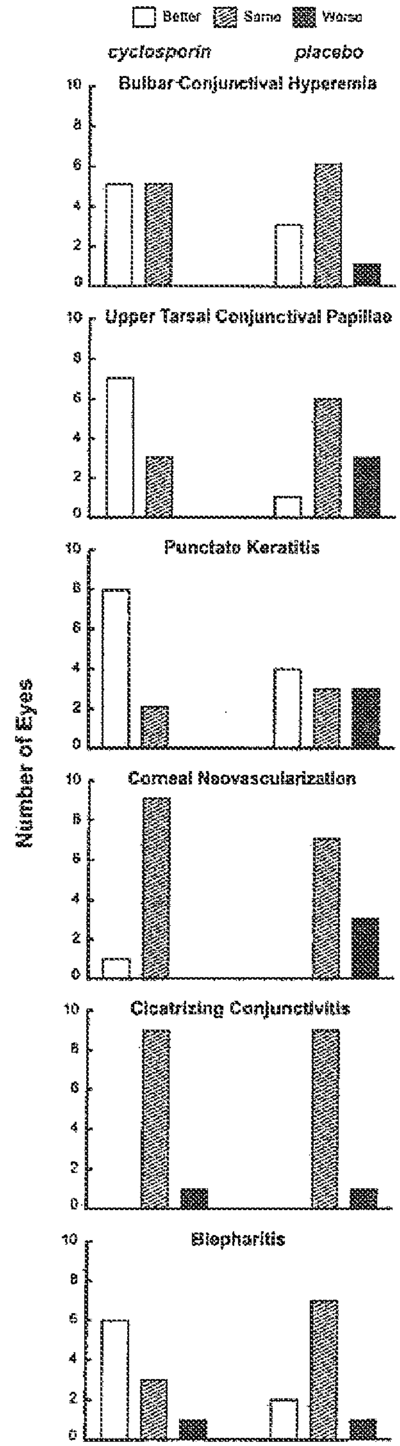

Figure 2. Signs of atopic keratoconjunctivitis.

Atopic keratoconjunctivitis is a rare form of severe ocular allergy with a pathogenesis of complex immune dysregulation and interplay of genetic, environmental, and psychologic factors. Several case series only, all from tertiary eye care centers, report the clinical and epidemiologic features of AKC, with no more than 55 patients at each individual center.[7,4,10,11] It is a chronic disease with symptoms lasting year-round, although up to 30% of patients report a seasonal influence.[3] Management is often difficult. Corneal complications are vision threatening and require immediate attention. Neovascularization, subepithelial haze, pannus, and pseudopterygium formation, seen in up to 60% to 70% of patients, are the most devastating complications of AKC, leading to blindness.[3,4] Importantly, approximately 30% of patients require corneal transplantation for visual or tectonic purposes.[4]

Cyclosporin A is most frequently used in solid organ transplantation. This unique and powerful immunomodulating agent has provided a new approach to therapy in autoimmune diseases and has been increasingly used as a molecular tool for investigating immune regulation. Cyclosporin A produces calcium-dependent, specific, reversible inhibition of transcription of interleukin-2, most notably in T helper lymphocytes.[5,6] This reduces the production of a range of cytokines, inhibiting the activation and/or maturation of various cell types, including those involved in atopy. Cyclosporin A's selective action on T helper lymphocytes and its weak myelotoxicity are the key advantages in the treatment of a large range of disorders in which immunoregulatory dysfunction is a suspected or proven etiologic factor. Oral cyclosporin A is an effective therapy in patients with severe inflammatory diseases refractory to standard treatment, such as ocular Behçet's syndrome, endogenous uveitis, psoriasis, atopic dermatitis, rheumatoid arthritis, active Crohn's disease, and nephrotic syndrome (reviewed in Faulds et al[12]). It is also effective in severe blinding AKC resistant to corticosteroids.[13] However, adverse effects, particularly nephrotoxicity, limit its widespread use as a systemic agent, particularly in non–life threatening diseases.

In the field of ophthalmology, topically applied cyclosporin A in various oil-based solvents was first used to inhibit experimental corneal allograft reaction in the early 1980s.[14–16] Later, the drug was found useful in patients with various inflammatory ocular surface disorders.[17–22] Topical cyclosporin A in oil has been used successfully in the treatment of vernal keratoconjunctivitis, yet another allergic eye disease with a T cell–dependent inflammatory mechanism, with dramatic clinical improvement.[23,24] In the one randomized, placebo-controlled trial involving steroid-dependent AKC, topical cyclosporin A 2% in maize oil was found to have a significant corticosteroid-sparing effect.[7] However, side effects, whether related to the oil solvent or the cyclosporin A itself, were common. Lid skin maceration, allergic reaction, and marked blurring of vision after drop instillation were attributed to the vehicle; however, intense stinging was a side effect of the cyclosporin A. Indeed, this is a well-known side effect that limits its clinical use.

Efficacy, safety, tolerability, and optimal dosing of a novel cyclosporin A oil-in-water emulsion formulation (Restasis) has been investigated in the treatment of moderate-to-severe dry eye disease in a phase III multicenter study.[25] Cyclosporin A ophthalmic emulsions (0.05% and 0.1%) were safe and well tolerated and significantly improved the ocular signs and symptoms of moderate-to-severe dry eye disease. No additional benefits were observed with the higher concentrations.

In this short-term, double-masked, randomized study, we used cyclosporin A 0.05% in an emulsion formulation in the treatment of patients with topical steroid–resistant AKC. Treated patients had greater improvement of both signs and symptoms of AKC than did the placebo group. No side effects attributed to cyclosporin A treatment were encountered. This formulation seems valuable in the treatment of topical steroid–resistant AKC. Its efficacy in the long-term treatment of patients with topical steroid–dependent or topical steroid–resistant AKC as a first-line agent should be considered, and warrants an additional, larger study.

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PRECLINICAL SAFETY STUDIES OF CYCLOSPORINE OPHTHALMIC EMULSION

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1. INTRODUCTION

Cyclosporine A (CsA) is an immunomodulator that inhibits T-cell mediated immunoreactivity.[1-4] In addition, data indicate that CsA may act as an anti-inflammatory, due to its phosphatase inhibition activity. Because of these actions, CsA ophthalmic emulsion formulation was developed by Allergan for treatment of keratoconjunctivitis sicca. Since the safety profile of this new formulation is very important for the clinical success of the drug, long-term safety studies were carried out as described below.

2. MATERIALS AND METHODS

2.1. Three-Month Ocular Study in New Zealand White Rabbits

Groups of ten rabbits/sex were treated with 0.05%, 0.2%, or 0.4% cyclosporine ophthalmic emulsion or the vehicle of 0.4% cyclosporine ophthalmic emulsion, three times daily, one drop in the left eye, for 3 months. The right eye served as an untreated control. Eight animals/sex/group were sacrificed after 3 months of treatment, and two animals/sex/group were allowed to recover for 1 month, after which they were sacrificed.

The parameters measured were: daily clinical observations; weekly body weight; weekly gross ocular observations; slit lamp biomicroscopy, ophthalmoscopy, hematology, and serum chemistry measured pretest, at the end of 1 month, 3 months, and recovery; end-of-study blood drug concentration (AUC, C_{max}, and C_{min}) using liquid chromatography-tandem mass spectrometry; gross observations at necropsy; organ weights; and histological evaluation of all tissues and organs.

Lacrimal Gland, Tear Film, and Dry Eye Syndromes 2
edited by Sullivan *et al.*, Plenum Press, New York, 1998

2.2. Six-Month Ocular Study in New Zealand White Rabbits

Groups of 15 rabbits/sex were treated with 0.05%, 0.2%, or 0.4% cyclosporine ophthalmic emulsion or vehicle of 0.2% cyclosporine ophthalmic emulsion three times/day, or 0.4% cyclosporine ophthalmic emulsion or the vehicle of 0.4% cyclosporine ophthalmic emulsion six times/day, one drop in the left eye, for 6 months. The right eye served as an untreated control. Ten animals/sex/group were sacrificed after 6 months of treatment, and five animals/sex/group were allowed to recover for 2 months, after which they were sacrificed.

The parameters measured were: daily clinical observations; weekly body weight; weekly gross ocular observations; slit lamp biomicroscopy and ophthalmoscopy pretest and at the end of 1 month, 3 months, treatment, and recovery; hematology and serum chemistry pretest, at the end of 3 months, treatment, and recovery; end-of-study blood drug concentration (AUC, C_{max}, and C_{min}) using liquid chromatography-tandem mass spectrometry; gross observations at necropsy; organ weights; and histological evaluation of all tissues and organs.

2.3. One-Year Ocular Study in Beagle Dogs

Groups of six beagle dogs/sex were treated with 0.4% cyclosporine ophthalmic emulsion or the vehicle of 0.4% cyclosporine ophthalmic emulsion six times/day, or 0.1% or 0.2% cyclosporine ophthalmic emulsion three times/day, one drop in the left eye, for 1year. The right eye served as an untreated control. Four animals/sex/group were sacrificed after 1 year of treatment, and two animals/sex/group were allowed to recover for 1 month, after which they were sacrificed.

The parameters measured were: daily clinical observations; weekly physical examinations; body weight and food consumption weekly during the first 3 months, monthly thereafter; weekly gross ocular observations; slit lamp biomicroscopy and ophthalmoscopy pretest and at the end of 1, 3, 6, 9, and 12 months and recovery; blood pressure and ECG pretest, at mid-study, and at end-of-study; hematology and serum chemistry pretest and at the end of 3, 6, 9, and 12 months and recovery; urine analysis pretest and at the end of 3, 6, and 12 months and recovery; blood drug concentration (AUC, C_{max}, and C_{min}) using liquid chromatography-tandem mass spectrometry during week 1 and at the end of study; gross observations at necropsy; organ weights; and histological evaluation of all tissues and organs.

This study was conducted at Corning Hazleton Laboratories in Vienna, VA.

3. RESULTS

3.1. Three-Month Ocular Study in New Zealand White Rabbits

Transient slight ocular discomfort and transient slight conjunctival hyperemia were observed in all treatment groups, including the vehicle group. There were no systemic adverse effects, and no compound-related histological changes in the eye or in any organs or tissues. Blood concentrations after the 0.05% treatment were generally below the limit of quantitation (0.1 ng/ml). The mean C_{max} values after 3 months of dosing with 0.2% and 0.4% cyclosporine ophthalmic emulsion were 1.48 and 0.721 ng/ml, respectively. (See Fig. 1.) AUC values for the 0.2% and 0.4% cyclosporine ophthalmic emulsion treatment groups were 4.52 and 4.28 ng•h/ml, respectively.

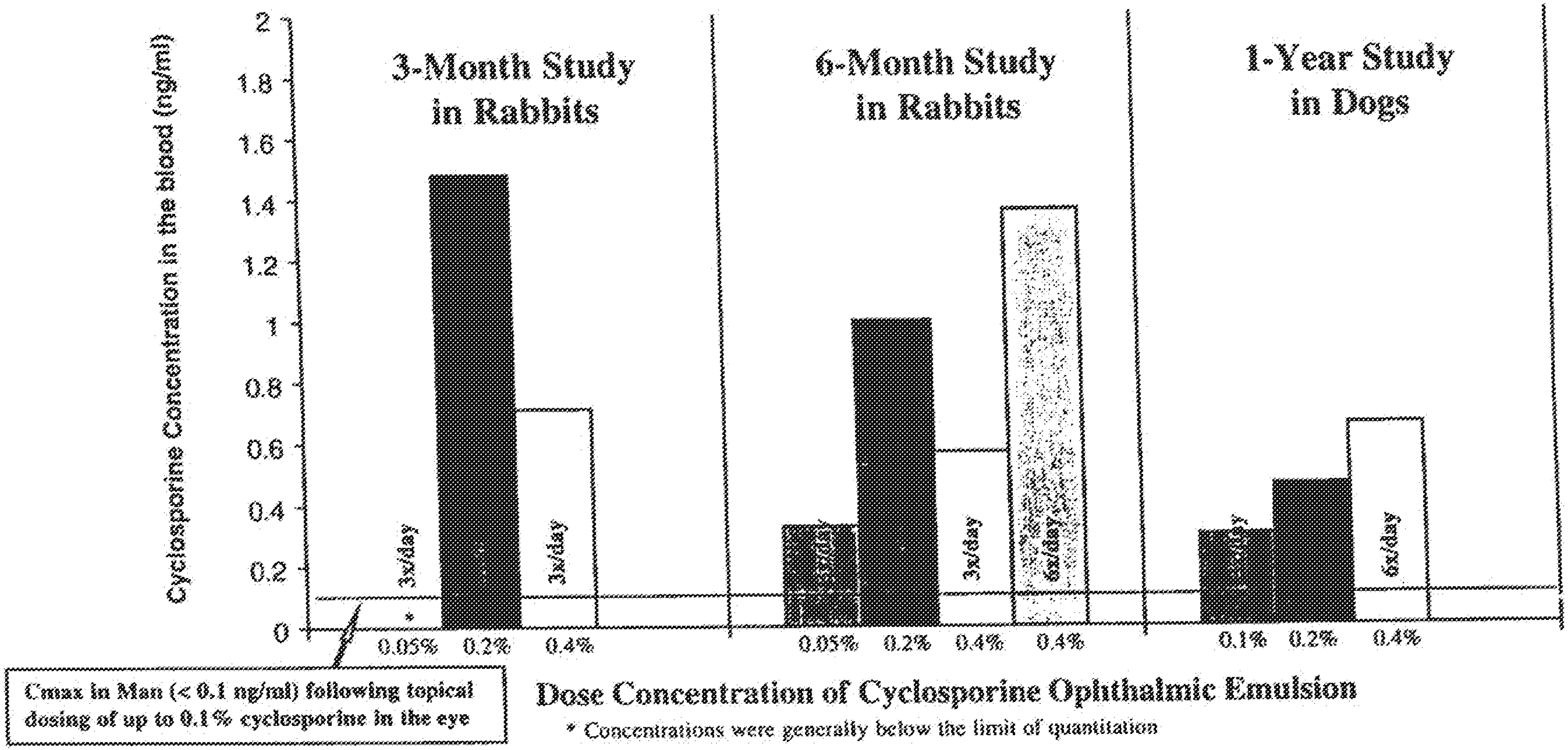

Figure 1. Mean peak blood cyclosporine concentration (C_{max}).

3.2. Six-Month Ocular Study in New Zealand White Rabbits

Transient slight ocular discomfort and transient slight conjunctival hyperemia were observed in all treatment groups, including the vehicle groups. There were no systemic adverse effects, and no compound-related histological changes in the eye or in any organs or tissues. The mean C_{max} values after 6 months of dosing with cyclosporine ophthalmic emulsion at concentrations of 0.05% TID, 0.2% TID, and 0.4% TID and six times daily were 0.328, 0.997, 0.570, and 1.36 ng/ml, respectively. (See Fig. 1.) AUC values for these groups were 3.48, 9.25, 6.85, and 16.7 ng•h/ml, respectively.

3.3. One-Year Ocular Study in Beagle Dogs

No ocular or systemic adverse effects were observed in any treatment group. There was a tendency toward an increased incidence of slight lacrimation of the treated eye in the group treated with 0.4% cyclosporine, which corresponds to the pharmacologic effect of this drug. There were no compound-related histological changes in the eye or in any organs or tissues. The mean C_{max} values after 1 year of dosing with cyclosporine ophthalmic emulsion at concentrations of 0.1% TID, 0.2% TID, and 0.4% six times daily were 0.299, 0.459, and 0.654 ng/ml, respectively. (See Fig. 1.) AUC values for these groups were 2.35, 3.39, and 9.55 ng•h/ml, respectively. There was no accumulation during this prolonged period of treatment.

4. DISCUSSION

In these animal studies, the concentrations and frequency of administration of cyclosporine ophthalmic emulsion represented an exaggerated dosing regimen compared with the proposed clinical regimen, which is once or twice daily administration of the maximum concentration of 0.1%. There were no clinical signs of toxicity in the eyes or systemically, and the histological examination of all tissues and organs did not reveal any compound-related findings.

The liquid chromatography-tandem mass spectrometry analytical method that was used to analyze cyclosporine concentrations in the blood is a significantly more sensitive method, with a limit of detection of 0.1 ng/ml, than the widely used radioimmunoassays and HPLC methods for cyclosporine, which have a limit of detection of 20 ng/ml. In the rabbit study and the dog study using concentrations of up to 0.4% cyclosporine ophthalmic emulsion six times daily, the majority of the individual peak blood drug concentrations were generally less than 1.5 and 1.2 ng/ml, respectively. This is at least 10 times higher than the peak blood drug concentrations in patients where the blood concentrations following ocularly administered 0.05 or 0.1% cyclosporine BID are less than 0.1 ng/ml.

5. CONCLUSION

The preclinical safety data in rabbits and dogs showed that cyclosporine ophthalmic formulations at concentrations up to 0.4% dosed as much as six times daily for up to 6 months in rabbits and up to 1 year in dogs did not show any topical or systemic toxicity. The systemic exposure to the cyclosporine formulation after ocular administration is very minimal. Therefore, cyclosporine ophthalmic formulation, at the proposed concentration, is judged to be safe for long-term application in humans.

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Safety assessment of cyclosporine ophthalmic emulsion in rabbits and dogs

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SUMMARY

Cyclosporine ophthalmic formulation offers potential therapy for dry eye diseases by inhibiting ocular surface immuno-inflammatory processes. To establish the safety of this new ophthalmic formulation, chronic studies were conducted in rabbits and dogs. Cyclosporine ophthalmic emulsion was tested topically in rabbits for 6 months at concentrations of 0.05% and 0.2% (3X/day) and 0.4% (3X and 6X/day), and in dogs for 1 year at concentrations of 0.1% and 0.2% (3X/day) and 0.4% (6X/day). All concentrations were very well tolerated topically in the dogs. In the rabbit study, transient slight ocular discomfort and conjunctival hyperemia were observed in all groups, including the vehicle control groups. There were no systemic adverse events and there were no microscopic changes in any tissues or organs. The systemic exposure was minimal even with the highest dosing regimen.

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INTRODUCTION

Cyclosporine administered orally has been extensively used for the prevention of graft rejection in organ transplants and more recently for autoimmune diseases. The mode of action of cyclosporine is to inhibit the T-cell mediated immunoreactivity. In addition, data indicate that cyclosporine acts as an anti-inflammatory, due to its phosphatase inhibition activity. Because of these data, cyclosporine ophthalmic emulsion was developed by Allergan for treatment of KCS with and without Sjögren's syndrome.

The local and systemic safety of this new ophthalmic drug has been evaluated in long term safety studies in rabbits and in beagle dogs.

MATERIALS AND METHODS

Six-Month Ocular Study in New Zealand White Rabbits: Groups of 15 rabbits/sex were treated with 0.05%, 0.2%, or 0.4% cyclosporine ophthalmic emulsion or vehicle three times/day or 0.4% cyclosporine ophthalmic emulsion or vehicle six times/day, one drop in the left eye for six months. The right eye served as an untreated control. Ten animals/sex/group were sacrificed after six months of treatment and five animals/sex/group were allowed to recover for two months after which they were sacrificed.

The parameters measured were: daily clinical observations; weekly body weight; weekly gross ocular observations; slit lamp biomicroscopy and ophthalmoscopy pretest and at the end of one month, three months, treatment, and the recovery period; hematology and serum chemistry pretest, at the end of three months, treatment and the recovery period, end-of-study blood drug concentration (AUC, C_{max}, and C_{min})[1] using liquid chromatography tandem-mass spectrometry, gross observations at necropsy and organ weights for all dose group animals, and histological evaluation of all tissues and organs of the vehicle and high-dose group animals and ocular tissues of all dose group animals.

One-Year Ocular Study in Beagle Dogs: Groups of six beagle dogs/sex were treated with 0.4% cyclosporine ophthalmic emulsion or the vehicle six times/day or 0.1% or 0.2% cyclosporine ophthalmic emulsion three times/day, one drop in the left eye for one year. The right eye served as an untreated control. Four animals/sex/group were sacrificed after one year of treatment and two animals/sex/group were allowed to recover for one month after which they were sacrificed.

The parameters measured were: daily clinical observations; weekly physical examinations; body weight and food consumption weekly during the first three months, monthly thereafter; weekly gross ocular observations; slit lamp biomicroscopy and ophthalmoscopy pretest and at the end of 1, 3, 6, 9, and 12 months and the recovery period; blood pressure and ECG during the pretest period and at mid-study and end-of-study; hematology and serum chemistry and urine analysis pretest and at the end of 3, 6, 9, and 12 months and the recovery period; blood drug concentration (AUC, C_{max}, and C_{min}) using liquid chromatography

[1] AUC = Area under the curve; Cmax = maximum blood concentration; Cmin = minimum blood concentration

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tandem-mass spectrometry during week 1 and at the end of study; gross observations at necropsy; organ weights; and histological evaluation of all tissues and organs of all animals.

RESULTS

Six-Month Ocular Study in New Zealand White Rabbits: Transient slight ocular discomfort and transient slight conjunctival hyperemia were observed in all treatment groups, including the vehicle groups. There were no systemic adverse effects and no compound-related histological changes in the eye or in any organs and tissues. The mean Cmax values after six months of dosing with cyclosporine ophthalmic emulsion at concentrations of 0.05% TID, 0.2% TID and 0.4% TID and six times daily were 0.328, 0.997, 0.570, and 1.36 ng/ml, respectively (See Figure 1). AUC values for these groups were 3.48, 9.25, 6.85, and 16.7 ng•hr/ml, respectively.

One-Year Ocular Study in Beagle Dogs: No ocular or systemic adverse effects were observed in any treatment group. There was an increased incidence of slight lacrimation of the treated eye in the group treated with 0.4% cyclosporine, which corresponds to the pharmacologic effect of this drug. There were no compound-related histological changes in the eye or in any organs and tissues. The mean Cmax values after one week of dosing with cyclosporine ophthalmic emulsion at concentrations of 0.1% TID, 0.2% TID and 0.4% six times daily were 0.207, 0.307, and 0.796 ng/ml, respectively (See Figure 1). AUC drug values after one week of dosing were not calculable (below the limit of quantitation) for the 0.1% dose group and 2.67 and 12.8 ng•hr/ml for the 0.2% and 0.4% dose groups,

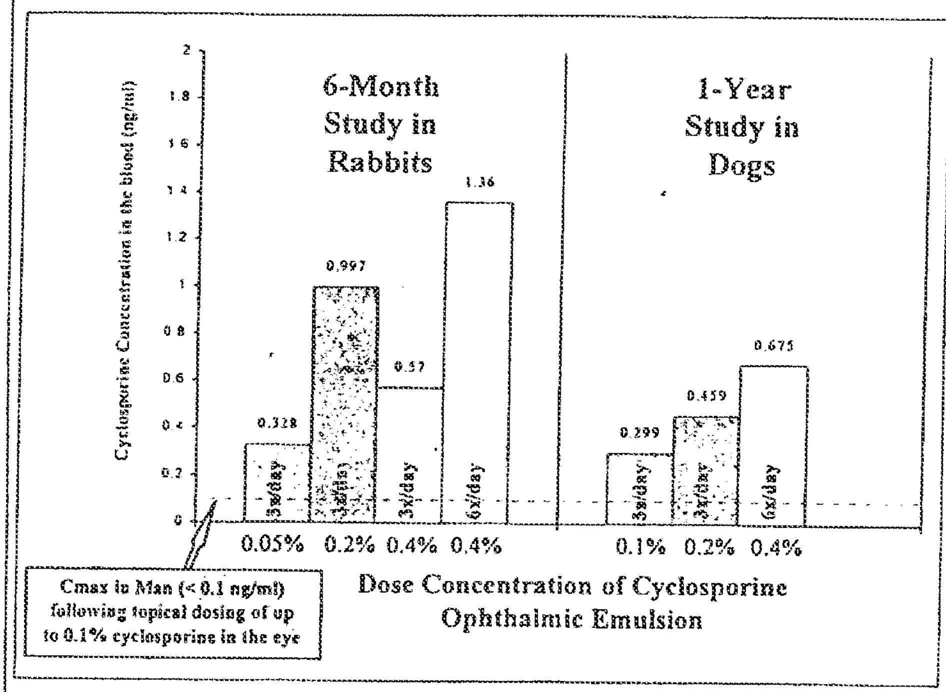

Figure 1
Mean Blood Cyclosporine Concentration (Cmax)

respectively. The mean Cmax values after one year of dosing with cyclosporine ophthalmic emulsion at concentrations of 0.1% TID, 0.2% TID and 0.4% six times daily were 0.299, 0.459, and 0.675 ng/ml, respectively. (See Figure 1) AUC values for these groups after one year are 2.35, 3.39, and 9.55 ng•hr/ml, respectively.

DISCUSSION

The concentrations and frequency of administration of cyclosporine ophthalmic emulsion in these studies represented an exaggerated dosing regimen compared to the proposed clinical regimen which is one drop in each eye once to twice daily of 0.1% cyclosporine ophthalmic emulsion. Nevertheless, no significant ocular or systemic toxicity was observed. In the dog study, the increased incidence of slight lacrimation in the treated eye in the high-dose group animals when compared to the control group animals is thought to be due to the lacrimomimetic effect of this drug.

Despite the fact that the animals were treated with high ophthalmic doses of cyclosporine, the systemic exposure to this drug was minimal. The blood drug concentrations found in these studies, however, were at least 10 times higher than those in patients treated with 0.05% or 0.1% cyclosporine ophthalmic emulsions BID. The majority of the individual whole blood drug concentrations were less than 1.5 ng/ml in rabbits and less than 1.2 ng/ml in dogs.

The liquid chromatography-tandem mass spectrometry analytical method which was used to analyze cyclosporine concentrations in the blood in these studies is a very sensitive method with a limit of detection of 0.2 ng/ml when compared to the widely used radioimmunoassays and HPLC methods for cyclosporine which have a limit of detection of 20 ng/ml.

CONCLUSION

The preclinical safety data in rabbits and dogs showed that cyclosporine ophthalmic formulations at concentrations up to 0.4% dosed up to six times daily for up to six months in rabbits and up to one year in dogs did not induce topical or systemic toxicity. The systemic exposure to the cyclosporine formulation after ocular administration is very minimal. Therefore, cyclosporine ophthalmic formulation, at the proposed concentration, is judged to be safe for long-term application in humans.

REVIEW ARTICLE

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A Practical Guide to the Management of Distal Ulcerative Colitis

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Contents

PTO-000490

Summary

This article reviews the role of corticosteroids, sulfasalazine and mesalazine (5-aminosalicylic acid, mesalamine), immunosuppressive agents and alternative novel drugs for the treatment of distal ulcerative colitis.

Short cycles of traditional, rectally administered corticosteroids (methylprednisolone, betamethasone, hydrocortisone) are effective for the treatment of mild to moderately active distal ulcerative colitis. In this context, their systemic administration is limited to patients who are refractory to either oral 5-aminosalicylates, topical mesalazine or topical corticosteroids. Of no value in maintaining remission, the long term use of either or topical corticosteroids may be hazardous. A new class of topically acting corticosteroids [budesonide, fluticasone, beclomethasone dipropionate, prednisolone-21-methasulphobenzoate, tixocortol (tixocortol pivalate)] represents a valid alternative for the treatment of active ulcerative colitis, and may be useful in the treatment of refractory distal ulcerative colitis.

Although there is controversy concerning dosage or duration of therapy, oral and topical mesalazine is effective in the treatment of mild to moderately active distal ulcerative colitis.

Sulfasalazine and mesalazine remain the first-choice drugs for the maintenance therapy of distal ulcerative colitis. Evidence exists showing a trend to a higher remission rate with higher doses of oral mesalazine. Topical mesalazine (suppositories or enemas) also is effective in maintenance treatment.

For patients with chronically active or corticosteroid-dependent disease, azathioprine and mercaptopurine are effective in reducing either the need for corticosteroids or clinical relapses. Moreover, they are effective for long term maintenance remission. Cyclosporin may be useful in inducing remission in patients with acutely severe disease who do not achieve remission with an intensive intravenous regimen. Existing data suggest that azathioprine and mercaptopurine may be effective in prolonging remission in these patients.

The role of alternative drugs for the treatment of distal ulcerative colitis and its different forms is reviewed. In particular data are reported concerning the effectiveness of 5-lipoxygenase inhibitors, topical use of short chain fatty acids, nicotine, local anaesthetics, bismuth subsalicylate enema, sucralfate, clonidine, free radical scavengers, heparin and hydroxychloroquine.

Ulcerative colitis is an inflammatory disease primarily affecting the colonic mucosa; the extent and severity of colon involvement are variable. In its most limited form it may be restricted to the distal rectum, while in its most extended form the entire colon is involved. However, more than half of the patients present with disease extending from the rectum to the splenic flexure. In this context, while ulcerative colitis may be classified into proctitis, proctosigmoiditis or left-sided colitis, the term 'distal colitis' is a working classification which implies that the inflammation is amenable to topical treatment as an alternative to, or in addition to, systemic treatment.

Despite significant progress made in the overall management of ulcerative colitis in the past decade, no new therapy has radically modified the principles of its treatment. This is probably the consequence of our substantial ignorance concerning the aetiology of the disease. As a result, the gastroenterologist treating patients with ulcerative colitis is entirely dependent on the outcome of clinical trials. The available drugs are useful in inducing remission of the disease, and some of them in preventing relapses, but there is no definite cure. However, the first priority in the treatment of ulcerative colitis is that the therapeutic knowledge we already have should be widely and efficiently applied. Al-

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and efficiently applied. Alternative treatments are being tested but most are unlikely to be generally available for many years.

This article reviews the role of the standard medical treatment of distal ulcerative colitis and the results of trials evaluating proposed new drugs which are potentially useful for alternative therapy, and provides practical guidelines for their correct and effective clinical application.

1. Aetiopathogenetic Background for the Pharmacological Treatment of Ulcerative Colitis

While the cause of ulcerative colitis remains unknown, certain features of this disease have suggested several areas of possible aetiological importance. These include familial or genetic, infectious, immunological and psychological factors. However, particular emphasis has been given to the study of either mucosal inflammation or immunological reactions.[1]

When the disease is active, the lamina propria of the mucosa becomes heavily infiltrated with a mixture of acute and chronic inflammatory cells. There is a predominant increase in mucosal immunoglobulin G production, evidence of complement activation, and activation of macrophages and T cells. This immunological activity is associated with the release of a vast array of cytokines, kinins, leukotrienes, platelet activating factor (PAF) and reactive oxygen metabolites. These mediators not only serve to amplify the immune and inflammatory response, but they also have direct effects on epithelial function, endothelial function (which may increase permeability and lead to ischaemia), and repair mechanisms, thus increasing collagen synthesis. In addition, many of the cytokines [interleukins- (IL) 1 and -6, tumour necrosis factor] will activate an acute phase response, resulting in fever and a rise in serum acute phase proteins. Some of the clinical features of acute ulcerative colitis may be explained by these mechanisms. It follows, therefore, that any treatment which is able to inhibit the activation of these immunological and inflammatory effector mechanisms is likely to lead to an improvement in the patient's symptoms and to a decrease in the inflammatory activity.

Glucocorticoids, sulfasalazine, mesalazine (5-aminosalicylic acid, 5-ASA) and immunosuppressive agents are able to inhibit many of these mechanisms and this may explain their effectiveness in the treatment of an inflammatory disease such as ulcerative colitis.

2. Definition of Activity Grade and Clinical Pattern of Disease

Two important criteria which should be used when choosing the most suitable therapeutic approach to patients affected by distal ulcerative colitis are: (a) grading the severity (mild, moderate or severe) and (b) defining the clinical pattern (intermittent chronic, chronically active or frequently relapsing) of the disease. Moreover, patient compliance is also a further important factor affecting the choice of therapy.

In ulcerative colitis, the activity of the disease is usually assessed primarily on the basis of clinical features. The Truelove and Witts classification[2] (table I) is the most widely used clinical activity index in gastroenterological practice. However, although clinically useful, these criteria do not allow sufficient discrimination for the purpose of clinical studies. Expansion of the clinical profile by Powell-Tuck et al.[3] has improved the design, implementation and results of clinical trials but lacks correlation with sigmoidoscopic appearance. Sutherland et al.[4] have proposed a useful disease activity index for ulcerative colitis including a rating

Table I. Classification of disease activity (moderate activity is activity intermediate between mild and severe) [adapted from Truelove & Witts[2]]

	Severe	Mild
Bowel movement frequency	≥6/day	≤4/day
Blood in stool	Mostly blood	Small amount
Fever	>37.5°C	None
Pulse rate	>90 beats/min	Normal
Haemoglobin	<75%	Normal
ESR	>30 mm/h	<30 mm/h

Abbreviation: ESR = erythrocyte sedimentation rate.

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Table II. Ulcerative colitis disease activity index[4]

Stool frequency	0	Normal
	1	1-2 stools daily more than normal
	2	3-4 stools daily more than normal
	3	>4 stools daily more than normal
Rectal bleeding	0	None
	1	Streaks of blood
	2	Obvious blood
	3	Mostly blood
Mucosal appearance	0	Normal
	1	Mild friability
	2	Moderate friability
	3	Exudation, spontaneous bleeding
Physician's rating of disease activity	1	Normal
	2	Mild
	3	Moderate
	4	Severe
Maximum score	13	

of clinical and endoscopic signs (table II). However, other clinical, endoscopical and histological indices are available for rating activity disease in ulcerative colitis.[5]

In 1963, Edwards and Truelove[6] characterised the course of ulcerative colitis. Despite the use of more modern medical therapies, the distribution of the clinical pattern shown by them is still accurate. After the first episode, most patients (approximately two-thirds) subsequently experience recurrent attacks. A small minority, from 7 to 15%, never achieve satisfactory remission and continue with symptoms to a greater or lesser degree (corticosteroid-resistant disease), while others have a corticosteroid-dependent disease. These different forms of disease must be considered when choosing the best pharmacological treatment for patients with distal ulcerative colitis, along with the knowledge that drugs may have several mechanisms of action and different toxicity profiles and routes of administration.

3. Pharmacology

With the diagnosis of distal ulcerative colitis lies the possibility of rectal drug administration, acting either towards inducing remission or preventing relapses. However, some drugs or formulations of the same pharmacological class as the rectally administered drugs also may be therapeutically effective even when systemically administered. The effectiveness of rectal formulations (enemas, suppositories and foam) of corticosteroids and mesalazine have been tested in clinical trials which have included patients affected by distal ulcerative colitis. In addition, however, the therapeutic effects of oral or parenteral formulations (corticosteroids, salicylates, immunosuppressants) have been evaluated in experimental studies including not only patients with extensive disease, but also patients with distal ulcerative colitis with variable grades of activity.

4. Corticosteroids

4.1 Older Corticosteroids

4.1.1 Systemic Corticosteroids

That corticosteroids have a beneficial effect on the course of ulcerative colitis was first suggested in the late 1940s.[7] Subsequent uncontrolled and controlled studies with adrenocorticotrophic hormone (ACTH), cortisone and hydrocortisone in the 1950s and 1960s confirmed that these drugs are effective in inducing remission of disease activity.[2,8]

Corticosteroids modify almost every part of the inflammatory response, including cell-mediated immunity and the production of inflammatory mediators such prostaglandins, leukotrienes, platelet activating factor and cytokines.[9,10] Their biological effects last longer than their plasma half-life.[11]

Although the efficacy of corticosteroids in acute ulcerative colitis has been proven, they should not be used for maintaining remission of the disease.[12-14] Prednisolone or hydrocortisone in equivalent doses are equally effective at controlling symptoms. Prednisolone is the preferred treatment because it has a lower mineralocorticoid effect at equipotent anti-inflammatory doses. The optimum initial dosage of oral prednisolone is 40 to 60 mg/day in a single morning dose.[15,16] Intravenous preparations of prednisolone are no longer

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available, but methylprednisolone 20mg 3 times daily can be given; this is slightly higher than the equivalent dose of prednisolone (methylprednisolone 4mg equals prednisolone 5mg).[17] Alternate-day therapy (prednisolone ≤30mg every other day), is useful when tapering the dose in patients who have achieved remission and are now asymptomatic, and in children, to prevent growth retardation. Preparations such as corticotrophin offer no advantage and are no longer used.

The main drawback of systemic corticosteroids is their potential to produce adverse effects, especially in association with prolonged use. Such effects include the development of cataracts, osteoporosis, bone necrosis, growth retardation in children, bodyweight gain, changes in mood, acne and, less commonly, hypertension or hyperglycaemia.[11] For these reasons, the goal of therapy is to achieve remission, taper the corticosteroid dose, and finally, discontinue their use.

4.1.2 Rectally Administered Corticosteroids

Rectal corticosteroid preparations have been the main treatment for attacks of mild to moderate distal ulcerative colitis since the 1950s, when Truelove et al.[18] performed a controlled trial of a rectal drip of hydrocortisone hemisuccinate (100 mg/day) versus placebo for 1 week in ulcerative colitis of varying severity. Since then, clinical trials[19,20] have shown that 3- to 4-week courses of this drug, as well as prednisolone-21-phosphate 20 to 40 mg/day and betamethasone 8 to 20 mg/day, improve both the symptoms and sigmoidoscopic appearance of distal ulcerative colitis in about two-thirds of patients with active disease.

In a placebo-controlled trial,[18] hydrocortisone enemas were shown to be of no value for weekend maintenance treatment of patients in clinical remission, although they were effective as short term therapy of active disease.

Short term corticosteroid therapy is associated neither with clinical evidence of systemic effects nor significant impairment of the response to ACTH stimulation. Occasionally, systemic effects develop, such as fluid retention, mooning of the face or acne, but these usually only occur in patients on long term treatment.[21] Although absorption of corticosteroids after topical administration is less than after oral administration, prolonged treatment with corticosteroid enemas may also produce adrenal suppression.[22]

Several galenic formulations of rectal corticosteroids have been developed: liquid enemas, foam enemas and suppositories. A 100ml liquid enema often reaches the splenic flexure,[23] foam enemas only occasionally reach as far as the descending colon,[24] while corticosteroids released from suppositories remain in the rectum.[25] Thus, for disease affecting the rectum and sigmoid, a foam enema is preferred. For proctitis alone, suppository administration may be sufficient. A liquid enema can be more effective for more extensive colitis, up to the splenic flexure. Clinical trials comparing rectal corticosteroids in liquid and foam formulations suggest that they are all equally effective; hydrocortisone and prednisolone suppositories have also been shown to be useful in treating idiopathic proctitis.[19,20]

Patients tend to dislike inserting medication through the anus and many, especially those with a poorly distensible rectum, have problems in retaining liquid enemas, particularly in the daytime. The foam enema is retained more easily, is more portable and is often preferred by patients.[26] In a study measuring the quality of life in ulcerative colitis, hydrocortisone foam enemas caused significantly less disturbance to work, outdoor and occupational activities and sexual relationships than prednisolone liquid enemas.[27]

4.2 Newer Topical Corticosteroids

The frequent adverse effects associated with the long term use of corticosteroids has prompted the development of a new group of agents. These newer agents may provide certain advantages over currently available corticosteroids by achieving equivalent or superior efficacy associated with a better adverse event profile.

The absence of toxicity in these newer topical agents relates to their low systemic bioavailability, which can be achieved in 3 ways: (a) extensive

Table III. Clinical trial results of newer rectally and orally administered corticosteroids in the treatment of active distal ulcerative colitis

Reference	No. of patients	Dosage (mg/day)	Route	Comparator	Treatment duration (wk)	Results
Prednisolone MSB						
McIntyre et al.[21]	40	20	PR	Prednisolone-21-phosphate	2	Idem
Fluticasone						
Angus et al.[30]	59	20	PO	Placebo	4	Idem
Hawthorne et al.[31]	205	20	PO	Prednisolone	4	Fluticasone < prednisolone
Tixocortol pivalate						
Hanauer et al.[32]	337	250	PR	Hydrocortisone	3	Idem
Friedman[33]	11	250 500	PR PR	No comparator		Reduction of mucosal inflammation
Beclomethasone (B)						
Kumana et al.[34]	9	0.5	PR	Betamethasone	2	Idem
Bansky et al.[35]	16	0.5	PR	Betamethasone	3	Idem
Van der Heide et al.[36]	18	30	PR	Prednisolone-21-phosphate	3	Prednisolone > beclomethasone
Halpern et al.[37]	32	0.5	PR	Betamethasone	4	Idem
Mulder et al.[38]	60	B 3mg + 5-ASA 2g	PR	B and 5-ASA alone	4	Combination better than either drug alone
Budesonide						
Danielsson et al.[39]	43	2	PR	Placebo	4	Budesonide > placebo
Matzen[40]	146	1, 2, 4	PR	Prednisolone	2 + 2	Idem
Lamers et al.[41]	63	2	PR	5-ASA	4 + 4	Idem
Tarpila et al.[42]	72	2	PR	Hydrocortisone	4	Idem
Bianchi Porro et al.[29]	88	2	PR	Methylprednisolone	4 + 4	Idem
Lofberg et al.[43]	100	2	PR	Prednisolone	8	Idem
Lemann et al.[44]	97	2	PR	5-ASA	4	Idem
Bayless et al.[45]	184	2	PR	Hydrocortisone and placebo	6	Budesonide > placebo and = hydrocortisone
Hanauer & Robinson[46]	233	0.5, 2, 8	PR	Placebo	6	2 and 8mg dosage > placebo
Lofberg et al.[47]	72	10	PO	Prednisolone	9	Idem

Abbreviations and symbols: 5-ASA = mesalazine; Idem = equally effective; MSB = metasulfobenzoate; PO = oral; PR = rectal; < = less effective than; > = more effective than.

first-pass metabolism in the blood by erythrocytes and in the liver; (b) lack of rectal absorption; or (c) both mechanisms. All these account for the low frequency of systemic effects of these agents. Moreover, these new drugs should have a high intrinsic glucocorticoid activity, high topical potency and good metabolic stability in the bowel. Prednisolone metasulfobenzoate, tixocortol (tixocortol pivalate), fluticasone propionate, beclomethasone dipropionate and budesonide are all considered to fulfil these requirements.

The results of trials (table III) with these nonsystemically active corticosteroids in enema or oral form, especially beclomethasone dipropionate (in dosages of 2, 3 and, rarely, 6 mg/day) and budesonide (1, 2, 4 and, rarely, 8 mg/day) have shown them to be as effective as conventional corticosteroids and mesalazine without the systemic corticosteroid adverse effects or suppression of the hypothalamic-pituitary-adrenal axis.[28,29]

In a very recent pilot study,[48] the efficacy of budesonide in an oral pH-modified release formu-

nt of active distal ulcerative colitis

Results
Idem
Idem
Fluticasone < prednisolone
Idem
Reduction of mucosal inflammation
Idem
Idem
Prednisolone > beclomethasone
Idem
Combination better than either drug alone
Budesonide > placebo
Idem
Idem
Idem
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Budesonide > placebo and = hydrocortisone
2 and 8mg dosage > placebo
Idem

) = oral; PR = rectal; < = less effective

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lation was evaluated for maintenance treatment in 14 patients with corticosteroid-dependent ulcerative colitis, during the reduction phase of conventional corticosteroids following an acute attack. Patients were treated with budesonide 3mg 3 times daily for 6 months. The primary investigation parameters were changes in the clinical activity index (CAI) and in the daily dose of corticosteroids. In 11 of 14 patients, CAI improved significantly and treatment with corticosteroids could be stopped. Three patients experienced relapse and needed further corticosteroid treatment. The average daily dose of corticosteroids and the mean value of the CAI before treatment with budesonide were significantly higher in the relapse group than in the remission group.

Unfortunately, plasma cortisol value and corticotrophin hormone stimulation tests were not carried out.

The main advantage of topically acting corticosteroids over traditional agents, especially budesonide, is the lack of systemic effect as well as of adrenal gland suppression. This is particularly useful for patients suffering from refractory distal ulcerative colitis or frequently relapsing disease, who need long term or high dose therapy. However, longitudinal studies, particularly addressing issues such as impact on bone turnover, will be required before the long term role for topical corticosteroids in the treatment of distal ulcerative colitis can be fully established.

5. Comparative Evaluation of Rectally Administered Corticosteroids and Alternative Treatments

Marshall and Irvine,[49] performed a meta-analysis of all reported randomised controlled trials with older and newer rectal corticosteroids, with the aim of critically examining the role of these agents in the treatment of active distal ulcerative colitis.

Pooled odds ratios (PORs) showed conventional rectal corticosteroids and rectal budesonide to be clearly superior to placebo, either for symptomatic and endoscopic improvement or for symptomatic and endoscopic remission.

In 7 trials, rectal mesalazine was significantly better than conventional rectal corticosteroids for inducing remission of symptoms, endoscopy and histology, with a POR of 2.42 [95% confidence interval (CI) 1.72-3.41)], 1.89 (95% CI 1.29-2.76), and 2.03 (95% CI 1.28-3.20), respectively.

Rectal budesonide was of comparable efficacy to conventional corticosteroids but produced less endogenous cortisol suppression.

Moreover, a cost comparison of rectal preparations was performed, showing that hydrocortisone 100mg and mesalazine 4g enemas are comparable in cost, while mesalazine 1g and 2g enemas cost considerably less. Budesonide enemas are marginally more expensive than hydrocortisone liquid enemas, and hydrocortisone foam costs slightly less.

6. Sulfasalazine and Mesalazine

Sulfasalazine is one of the major advances in the treatment of ulcerative colitis and represents the mainstay of maintenance therapy for patients in remission. On the suspicion of a bacterial cause of ulcerative colitis, sulfasalazine was developed with the aim of delivering a compound consisting of an antibacterial, sulfapyridine, attached to an anti-inflammatory agent (mesalazine), to the mucosa of patients with the disease. The antibacterial and anti-inflammatory components are linked by an azobond that is split by bacteria in the colon.

Misiewicz et al.[50] in 1965 were the first to show that oral sulfasalazine, in a dose of 0.5g 4 times a day for a follow-up period of 1 year, was significantly more effective than placebo in sharply reducing the ulcerative colitis relapse rate. Overall, the 1-year relapse rate was about 30% with sulfasalazine and 75% with placebo.

Since then, other controlled trials have confirmed the efficacy of sulfasalazine in preventing ulcerative relapses, and more and more patients the world over are continuously treated with this drug. A recent meta-analysis including only placebo-controlled trials showed an increase in the therapeutic advantage (expressed as a remission rate) of

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Table IV. Oral formulations of mesalazine

Formulation	Drug delivery
Azo-bond	
Olsalazine	Colon
Ipsalazine	Colon
Balsalazine	Colon
Mesalazine	
'Salofalk'	Ileum – colon
'Claversal'	Ileum – colon
'Asacol'	Distal ileum – colon
'Pentasa'	Small bowel – colon

sulfasalazine over placebo from 22% to 42% when comparing 6-month to 12-month trials, suggesting a decline in the efficacy of placebo, while the efficacy of sulfasalazine remained constant.[51]

Adverse effects occur in 10 to 40% of patients receiving sulfasalazine.[52] Dose-related adverse effects include vomiting, anorexia, dyspepsia, headache, skin discoloration and decreased sperm count. Idiosyncratic responses including hypersensitivity reactions such as skin rash, bronchospasm, haemolytic anaemia and hepatocellular changes occur rarely in patients treated with sulfasalazine. Moreover, albeit with a low incidence, sulfasalazine treatment may be associated with possibly lethal agranulocytosis. Most of these effects are attributed to the sulphonamide component of sulfasalazine.

The demonstration by Azad Khan et al.[53] in 1977 that mesalazine is the active therapeutic moiety of sulfasalazine and acts topically on the colonic mucosa led to the development of new oral mesalazine formulations which do not contain the sulpha component (table IV). In some of these, sulfapyridine has simply been replaced by another carrier and linked to the mesalazine molecule by a nitrogen bridge: (a) another mesalazine molecule in olsalazine, (b) 4-aminobenzoylglycine and 4-amino-benzoyl-β-alanine, linked respectively in ipsalazine and balsalazine. As in the case of sulfasalazine, however, mesalazine is released into the colon by these formulations only after bacterial splitting of the nitrogen bond. To obviate the need for bacterial splitting, other formulations have been developed in which mesalazine is not linked to another carrier.

In these gastrointestinal formulations, mesalazine is coated either with a semipermeable ethyl cellulose membrane ('Pentasa'), or, as in the case of mesalazine, with an acrylic resin ('Eudragit S', 'Asacol', 'Eudragit L', 'Salofalk' or 'Claversal'), which retards the release of the active molecule, especially in the colon.

Two recent meta-analyses by Sutherland et al.[54,55] assessed the effectiveness of oral mesalazine compared with placebo or sulfasalazine for the treatment of active ulcerative colitis and for the maintenance of remission (see section 6.1).

6.1 Oral Mesalazine and Sulfasalazine in Active Ulcerative Colitis

In the earlier meta-analytic review,[54] 8 trials comparing 1007 patients examined oral mesalazine compared with placebo for acute mild-to-moderate ulcerative colitis. The POR for complete remission combining all mesalazine preparations with all doses was 2.02 (fig. 1). A dose-dependent trend was apparent, with a lack of statistically significant difference from placebo for doses of mesalazine less than 2 g/day. The POR for patients

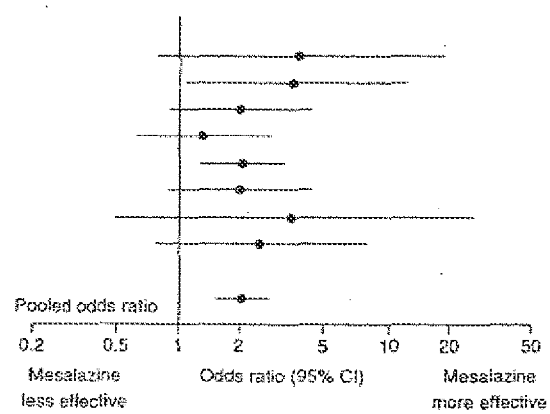

Fig. 1. Odds ratios together with 95% confidence intervals (CI) for 8 trials comparing oral mesalazine with placebo in the treatment of active ulcerative colitis. Values greater than 1 indicate a therapeutic advantage compared with placebo (from Sutherland et al.,[54] with permission).

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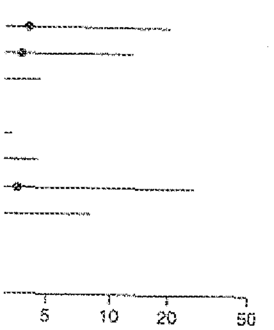

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receiving at least 2 g/day of mesalazine showed improvement compared with placebo. No significant differences in response could be shown between any of the mesalazine preparations.

Eight studies, involving 553 patients, compared mesalazine with sulfasalazine for the acute treatment of ulcerative colitis. The POR for complete remission, after combining all mesalazine formulations and comparing them with sulfasalazine, was 1.15 (fig. 2). No evidence existed for a dose-response relationship, and no significant differences in efficacy were found between the various mesalazine preparations compared with sulfasalazine.

In the more recent review,[55] in which the inclusion of more recent studies increased the global number of patients by >1500 (total n = 4373), giving the potential for greater statistical power, the results of the original meta-analysis have been largely confirmed. However, some aspects that differ from the previous work have been shown. In particular, the major distinction between the 2 reviews was the analysis of the data concerning complete remission separately from those concerning improvement/remission. Moreover, different quality assessment criteria were also used. On the basis of an intention-to-treat principle, the outcome of interest in the treatment of active disease was the failure to induce global/clinical remission, global/clinical improvement, endoscopic remission or endoscopic improvement.

Of the 19 trials, involving 2032 patients, 9 were placebo controlled, and 10 compared mesalazine with sulfasalazine. All the results were expressed as odds ratios, with a value of <1.0 indicating a beneficial effect of mesalazine treatment over the comparison therapy.

Mesalazine was superior to placebo with regard to all measured outcome variables. For the failure to induce global/clinical improvement or remission, the POR was 0.39 (95% CI 0.29-0.52). A dose-response trend for mesalazine was also observed. When mesalazine was compared with sulfasalazine, the POR was 0.87 (95% CI 0.63-1.20) for the failure to induce global/clinical improvement or remission, and 0.66 (95% CI 0.42-1.04) for the failure to induce endoscopic improvement. Thus, as in the previous meta-analysis, there was a trend in favour of a slight benefit for the newer mesalazine formulations over sulfasalazine in the induction of global/clinical and endoscopic improvement (including remission) in active disease. The incidence of adverse events and withdrawals due to the mesalazine formulations did not significantly differ from that associated with placebo, and was less than that observed among the patients treated with sulfasalazine.

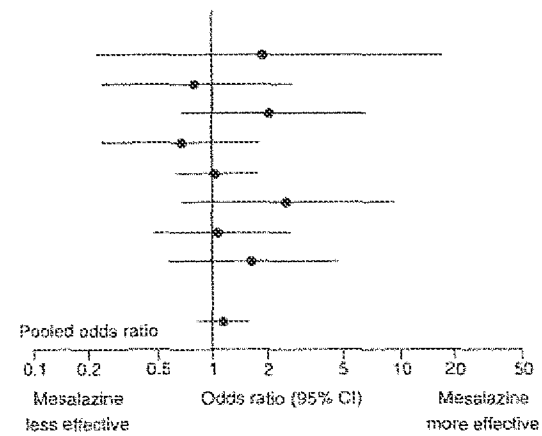

Fig. 2. Odds ratios together with 95% confidence intervals (CI) for 8 trials comparing oral mesalazine with sulfasalazine in the treatment of active ulcerative colitis. Values greater than 1 indicate a therapeutic benefit for mesalazine compared with sulfasalazine (from Sutherland et al.,[54] with permission).

6.2 Quality of Life for Distal Ulcerative Colitis Patients Treated with Oral Mesalazine

In an 8-week, randomised, dose-response, placebo-controlled, double-blind, multicentre trial,[56] quality of life was evaluated in 374 patients with active ulcerative colitis using controlled release mesalazine capsules ('Pentasa') at 1, 2 and 4 g/day versus placebo. 156 patients had distal ulcerative colitis. Function-related quality-of-life parameters were assessed in this study, including 5 pertinent symptoms and 7 general life capabilities. All the parameters were recorded using a 10cm vi-

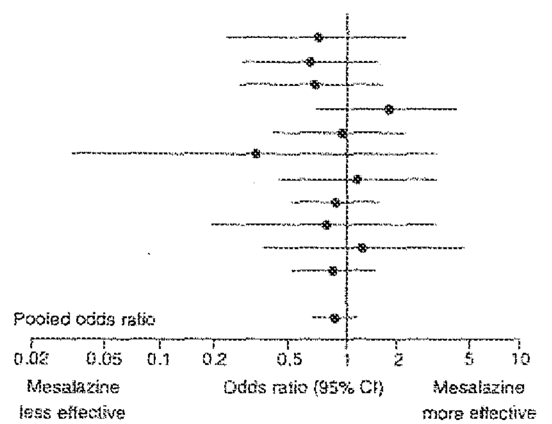

Fig. 3. Odds ratios together with 95% confidence intervals (CI) for 11 clinical studies comparing oral mesalazine with sulfasalazine in the maintenance or remission of ulcerative colitis. Values of less than 1 indicate a benefit for sulfasalazine compared with mesalazine (from Sutherland et al.,[54] with permission).

sual analogue scale except trips to the toilet, which were recorded in the patients' diaries. Mesalazine at 2 and 4 g/day was significantly superior to placebo in improving each of the 12 quality-of-life parameters ($p < 0.05$), indicating that this oral mesalazine formulation significantly enhanced the quality of life for patients with active ulcerative colitis.

6.3 Oral Mesalazine and Sulfasalazine in Ulcerative Colitis in Remission

In the original meta-analysis,[54] 11 trials, involving 1153 patients, compared mesalazine with sulfasalazine and placebo for the maintenance therapy of ulcerative colitis. Ten of 11 trials defined clinical response as maintaining remission during a 12-month period. The POR for these studies was 0.85 (fig. 3). The POR for trials in which high dosages of mesalazine were used was not significantly different from that found in the trials using low dosages (0.89 *vs* 0.82) [fig. 3]. Thus, no significant difference exists among the various mesalazine formulations, and none of these was more efficacious than sulfasalazine. However, it must be observed that the relapse rates reported in these studies differed greatly, ranging from approximately a minimum of 20% to a maximum of 50% for mesalazine-treated patients, compared with 12 and 55%, and 24 and 62%, for patients treated with sulfasalazine and placebo, respectively.

Many factors may explain these discrepancies such as differences in patient population and selection (most patients included had remission of a very short duration which, in some cases, was only clinically evaluated, but not endoscopically and histologically examined), length of follow-up (4 to 6 months in some studies, 12 months in others), dosage of maintenance drug (from 0.8 to 4 g/day), relapse definition and success parameters for treatment.

In the more recent review,[55] 16 studies were evaluated involving 2341 patients. Only 4 were placebo-controlled; the remaining 12 trials compared mesalazine with sulfasalazine. Here again, an odds ratio with a value of <1.0 indicated a beneficial effect of mesalazine over the comparison therapy.

The POR for the failure to maintain clinical or endoscopic remission for mesalazine versus placebo was 0.48 (95% CI 0.35-0.65) and versus sulfasalazine, 1.29 (95% CI 1.06-1.57) at 6 months and 1.15 (95% CI 0.89-1.50) at 12 months. Compared with the original meta-analysis, this review

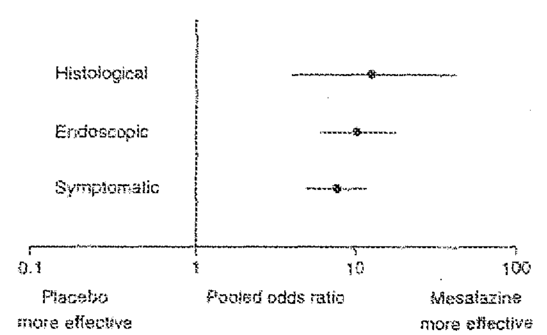

Fig. 4. Odds ratios together with 95% confidence intervals (CI) for 4 of 5 trials comparing rectal mesalazine with placebo in which data for endoscopic and histological improvement were provided. The respective pooled odds ratios were 10.04 (95% CI: 5.72-17.61) and 10.31 (95% CI: 5.85-18.18) [from Marshall & Irvine,[57] with permission].

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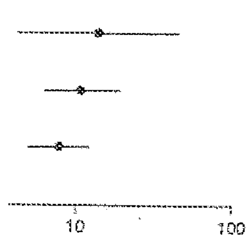

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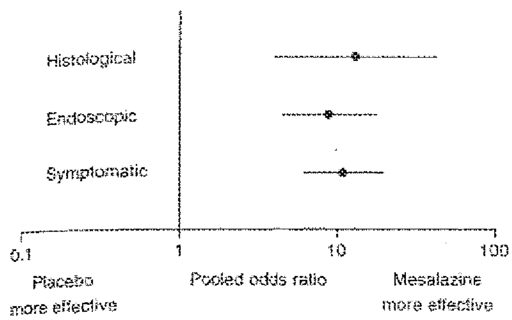

Fig. 5. Odds ratios together with 95% confidence intervals (CI) for 5 studies comparing rectal mesalazine with placebo, in which symptomatic, endoscopic and histological remission rates were reported. The respective pooled odds ratios were 10.37 (95% CI: 5.72-18.80), 8.23 (95% CI: 4.08-16.58) and 12.47 (95% CI: 3.75-41.43) [from Marshall & Irvine,[57] with permission].

with the analysis of trials involving an additional 1188 patients showed that sulfasalazine has a modest but statistically significant benefit over mesalazine, at least for up to 48 weeks. However, these results must be interpreted with caution considering that the meta-analysis included all trials, regardless of whether relapse was defined in terms of clinical or endoscopic criteria. Thus, in a patient population selected for tolerance to sulfasalazine, there is insufficient evidence to confirm the benefit of mesalazine over sulfasalazine for maintenance therapy.

6.4 Rectal Salicylates (Aminosalicylic Acid, Mesalazine)

A meta-analysis of reported randomised, controlled trials assessing topical aminosalicylic acid and mesalazine, in enema or suppository formulation, for the treatment of distal ulcerative colitis, has been published recently.[57] In this analysis, in mild to moderately active disease, the proportion of patients who improved symptomatically with rectal mesalazine (in dosages varying from 1 to 4 g/day) ranged from 60 to 94%, compared with 14 to 42% in the placebo groups (fig. 4). Rates of symptomatic remission varied between 31 to 80% in mesalazine-treated groups and 7 to 11% in those receiving placebo (fig. 5). Comparing mesalazine with placebo, symptomatic improvement and remission rates were consistently higher than the endoscopic rates which were, in contrast, more favourable than the rates calculated using histology. In the trials where mesalazine was compared with prednisolone, the PORs were low for both symptomatic improvement (1.39) and remission (1.10) (fig. 6). No clear dose-response relationship was identified. In the studies evaluating rectal 4-aminosalicylic acid, symptomatic improvement rates ranged from 71 to 100% in the treatment of active ulcerative colitis. Rates for induction of remission ranged from 38 to 85%.

In quiescent disease, rectal mesalazine was compared with placebo or with an oral mesalazine preparation. Dosage regimens varied considerably among the studies (from 1 to 4 g/day).

The rate of remission maintenance ranged between 54 to 80% in patients receiving the active treatment and 15 to 20% in those treated with placebo.

The long term use of mesalazine enemas is also effective in inducing remission in patients with left-sided ulcerative colitis which is unresponsive to or intolerant of conventional therapy, with an 80% remission rate by 34 weeks, thus allowing patients to reduce or discontinue glucocorticoid treatment.[58]

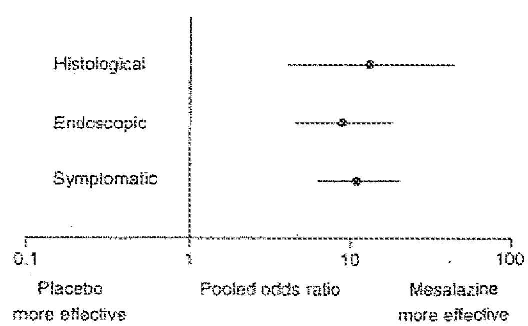

Fig. 6. Odds ratios together with 95% confidence intervals (CI) for 2 trials comparing rectal aminosalicylic acid and mesalazine with rectal prednisolone. The pooled odds ratios were low for both symptomatic improvement (1.39; 95% CI: 0.73-2.66) and remission (1.10; 95% CI: 0.51-2.38). The 95% CI included an odds ratio of 1 (no improvement) [from Marshall & Irvine,[57] with permission].

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A double-blind study, using enemas containing mesalazine 1, 2 or 4g or placebo for a 1-month treatment period, demonstrated that mesalazine 1g (in a 100ml enema) is a sufficient dose for patients with a mild to moderate attack of ulcerative colitis.[59] This was further confirmed by a recent trial in which a high percentage of clinical and endoscopic improvement or remission (88.9 and 74.1%, respectively) was obtained in patients with distal active ulcerative colitis, with a low dosage of mesalazine enemas (1 g/day), after only a 3-week treatment period.[60]

Reports of adverse events coming from topical mesalazine treatment varied substantially from study to study. In many trials reporting adverse reactions (anal canal irritation, headache, facial erythema, hair loss and peripheral oedema) their relative frequency was not recorded, but all were classified as minor.[57]

6.5 Quality of Life for Patients with Distal Ulcerative Colitis Treated with Rectal Mesalazine

In an effort to increase the benefits of mesalazine rectal therapy, improving either the delivery of a high amount of the drug to the diseased colonic tract, or quality of life and patient acceptance, several galenic formulations of rectal mesalazine are available in clinical practice for patients with distal ulcerative colitis.

The efficacy, tolerance and acceptance of a mesalazine colonic foam were compared in 233 patients with active distal ulcerative colitis from 12 outpatient clinics in Italy, against those of a liquid enema formulation of the same drug.[61] Analysis of the data collected showed that a higher proportion of patients receiving foam reported good acceptance (81%), in comparison with enema treatment (49%) ($p < 0.01$). Among patients treated with foam, 81% preferred the new formulation over the previous use of enema, because foam was more comfortable, more practical, easier to retain and caused less interference with daily life.

In another Italian study,[62] the efficacy, tolerance and acceptance of a new mesalazine 1g suppository once daily versus a mesalazine 500mg suppository twice daily, in patients with proctitis and proctosigmoiditis, were compared in a 4-week investigator-blind study. After 4 weeks of treatment no statistically significant difference was found between the 2 treatment groups in terms of efficacy. On the other hand, the patient evaluation for tolerability and practicality, measured by a score of patients' opinions, showed that mesalazine 1g was significantly superior to mesalazine 500mg twice daily, resulting in a more practical approach with a minor interference with the patients' daily activities.

A new rectal preparation of mesalazine in gel, without the addition of propellent gas, for topical treatment in distal ulcerative colitis has been developed recently in Italy. The efficacy, safety and patient acceptability of this new mesalazine 2g enema at night was compared with mesalazine 2g foam enemas, in the treatment of mild to moderate distal ulcerative colitis in a 4-week prospective, randomised, investigator-blind study.[63] After 4 weeks, no statistical difference was observed between the 2 formulations in terms of remission rate (76% for the gel enema versus 69% for the foam). Patient evaluation for tolerability and acceptability of the therapy showed that the new mesalazine gel formulation was significantly better tolerated than the foam because it was easier to retain and caused less abdominal bloating and discomfort during administration.

7. Combined Oral and Rectal Treatment with Salicylates

Very few studies have compared the efficacy and safety of combined oral and rectal treatment versus oral therapy alone with sulfasalazine and mesalazine, in patients with distal ulcerative colitis.

Kam et al.[64] compared mesalazine suspension enema, 4g at night, with oral sulfasalazine 1g 4 times daily, in a 6-week, double-blind, double-dummy, multicentre study involving 37 patients with active mild to moderate distal ulcerative colitis. At the end of the study, rectally administered

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mesalazine was as effective as oral sulfasalazine, but was associated with fewer and milder adverse events. Patients treated with mesalazine reported improvement earlier than those treated with oral sulfasalazine.

The efficacy of a combination of oral and rectal mesalazine for the maintenance treatment of ulcerative colitis was evaluated in a double-blind, randomised clinical trial involving 72 patients (63 with distal ulcerative colitis) with a history of 2 or more relapses during the past year or who had achieved remission in the past 3 months.[65] Patients enrolled in the study were assigned to: (a) combined therapy with mesalazine tablets 1.6 g/day and mesalazine enemas 4g/100ml twice weekly, or (b) oral therapy with mesalazine tablets 1.6 g/day and placebo enemas twice weekly, for 12 months.

Upon completion of the study, relapse occurred in 13 of 33 patients in the combined treatment group versus 23 of 36 in the oral treatment group (39 *vs* 69%, p = 0.036). No significant adverse effects related to treatment were observed in either group, and patient acceptance of the combined treatment scheme was good. A simplified pharmacoeconomic analysis showed that this form of combined treatment could have a favourable cost effectiveness ratio compared with the oral treatment alone.

8. Topical Combination Therapy with Mesalazine and Corticosteroids

Although the use of a topical combination therapy with mesalazine and corticosteroids is frequent in clinical practice, to date only 1 study has evaluated the effect of this treatment. Mulder et al.[38] performed an interesting clinical study suggesting that the topical combination of beclomethasone dipropionate with mesalazine may consistently increase the proportion of patients achieving clinical, endoscopic and histological improvement in the short term treatment of distal ulcerative colitis.

9. Immunosuppressive Agents

9.1 Azathioprine and Mercaptopurine

The rationale for the use of immunosuppressive agents in the treatment of ulcerative colitis stems from observations implicating immunomechanisms in the pathogenesis of the disease.

The most widely used drug is azathioprine, which is metabolised to mercaptopurine.[66] Little information is available about the mode of action of azathioprine in inflammatory bowel disease. The drug has an effect on lymphoid cell populations,[67,68] and some of these changes are slow to occur, paralleling the long duration of treatment required to produce a clinical effect.

The drug should be given at a dosage of 2 mg/kg/day and several months of treatment are required for it to have its maximum effect. Bone marrow depression, manifested as reversible leucopenia, is one of the most frequent adverse effects of azathioprine treatment. To avoid leucopenia a blood count should be done every 2 weeks in the first month and thereafter monthly.[69] Falls in the white cell count reverse on stopping the drug. About 6% of patients cannot tolerate the drug because of nausea, a flu-like syndrome, fever or pancreatitis. A significant increase in malignancy has been reported in patients with renal transplants taking this drug. However, in ulcerative colitis the dose used, the use of associated immunosuppressive drugs, and the changes in the immune system are different from those in transplants and malignancy does not seem to be increased.[70]

Therapeutically effective in controlling symptoms for patients who have frequent relapses as well as in those with chronically active disease which flares up when corticosteroids are reduced,[71,72] azathioprine and mercaptopurine have also shown their effectiveness in maintaining remission of ulcerative colitis.

In a British multicentre trial, Hawthorne et al.,[73] randomising 67 patients in full remission while taking azathioprine for 2 months or more to either continue azathioprine treatment or take placebo, showed that the 1-year relapse rate was 36%

(12/33) for patients continuing azathioprine and 59% (20/34) for those taking placebo (p = 0.04). These results show that azathioprine maintenance treatment in ulcerative colitis is beneficial for at least 2 years if patients have achieved remission while taking the drug. Similarly, high relapse rates were found in patients who discontinued active treatment by George et al.,[74] who retrospectively evaluated the long term outcome of patients with refractory ulcerative colitis treated with mercaptopurine.

It should be emphasised that much of the information about the indication for azathioprine or mercaptopurine in treatment comes from a few open, uncontrolled studies rather than double-blind and controlled trials. Moreover, not all patients included in these clinical studies had distal ulcerative colitis. However, considering that ulcerative colitis can definitely be cured by proctocolectomy, and that this solution is particularly indicated for patients who have had universal disease for 10 years or longer and who theoretically are at risk for cancer, patients with refractory left-sided colitis may be a specific therapeutic subgroup in whom proctocolectomy may be avoided, and treatment with one of these 2 drugs may be indicated.

9.2 Cyclosporin

Cyclosporin is a neutral, lipophilic cyclic peptide produced by the soil fungus *Tolypocladium inflatum gams*, which interrupts the cellular immune response by blocking the production of IL-2 by T helper lymphocytes.[75] The role of cyclosporin in the treatment of inflammatory bowel disease has been reviewed in detail recently.[76]

A recent small, controlled clinical study[77] showed the efficacy of continuously infused intravenous cyclosporin 4 mg/kg/day in patients with severe ulcerative colitis (both left-sided and pancolonic) unresponsive to intravenous corticosteroids. Cyclosporin induced remission in 80% of patients who would otherwise have undergone immediate colectomy. The use of intravenous cyclosporin must be balanced with the danger of epileptic fits in hypocholesterolaemic patients, the very real risk of opportunistic infections and irreversible impairment of renal function.[76]

The role of oral cyclosporin in the maintenance treatment of patients with ulcerative colitis who were initially treated with intravenous cyclosporin due to the refractory nature of their disease to standard medical treatment is controversial. In the study by Lichtiger et al.,[77] 4 of 9 patients (44%) successfully treated with intravenous cyclosporin underwent colectomy during a 6-month follow-up period of oral drug at a dosage of 8 mg/kg/day. The addition of mercaptopurine to the regimen of all responders to intravenously administered cyclosporin was recommended.

In a subsequent study of the same group,[78] 18 of 29 cyclosporin responders were placed on mercaptopurine, and 11 were not. During a 92-week follow-up period, 78% of the patients treated with mercaptopurine avoided colectomy compared with 36% of the patients who did not receive that drug. Similar results were recently reported with azathioprine treatment in a small series of patients with intravenous cyclosporin–induced remission of ulcerative colitis.[79]

Cyclosporin enemas have been employed in the treatment of resistant proctitis. Although open studies[76] have reported its successful treatment, in the only placebo-controlled double-blind trial, published in 1994,[80] cyclosporin enemas at a daily dosage of 350mg for 1 month were not effective in the treatment of 40 patients with mild to moderate active left-sided ulcerative colitis. No toxicity was observed and whole blood cyclosporin concentrations were undetectable in all but 2 patients.

9.3 Methotrexate

Methotrexate is an antimetabolite folic acid inhibitor with both immunosuppressor and anti-inflammatory activity. The drug also has molecular homology to IL-1 and interferes with the inflammatory action of IL-2.[81]

In an open trial of parenteral methotrexate,[82] 75% of patients with refractory active inflammatory bowel disease (14 with Crohn's disease and 7 with ulcerative colitis) improved over 12 weeks

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Table V. Alternative drugs for the treatment of distal ulcerative colitis

5-Lipoxygenase selective inhibitors
Topical short chain fatty acids
Nicotine
Local anaesthetics
Bismuth subsalicylate enema
Clonidine
Free radical scavengers
Heparin

with intramuscular methotrexate 25mg weekly. Improvement was reflected by activity indices and steroid requirement. However, in a very recent double-blind study, methotrexate at a weekly oral dose of 12.5mg was not found to be better than placebo in the induction or maintenance of remission in patients with chronic steroid-dependent ulcerative colitis.[83]

The role of oral methotrexate and the value of this drug in the maintenance treatment of ulcerative colitis is questionable. In an open study of low dose therapy in patients with active ulcerative colitis, 3 of 8 patients had a partial response to oral methotrexate 15mg weekly at 18 weeks, but the treatment failed in the long term in all patients.[84]

Adverse effects of methotrexate include post-injection nausea, diarrhoea or stomatitis in 10% of patients, minor leucopenia or elevation of liver function test values, brittle nails and occasional hair loss. Occasionally, hypersensitivity pneumonitis may occur. Moreover, the potential long term risk of liver fibrosis must be considered when patients are placed on this treatment.

10. Alternative Drugs for the Treatment of Distal Ulcerative Colitis

In recent years several reports have been published that have outlined the therapeutic potential of alternative and sometimes experimental forms of drug therapy[85] (table V).

10.1 5-Lipoxygenase Selective Inhibitors

The first dedicated enzyme in leukotriene synthesis is 5-lipoxygenase. New selective lipoxygenase inhibitors have been used in the past with variable success and are still being evaluated. Two double-blind trials[86,87] have shown that zileuton, a novel inhibitor of 5-lipoxygenase, at variable doses (600 to 800mg) and regimens (once, twice and 4 times daily), appeared to be of some efficacy in the treatment of active distal ulcerative colitis. However, 2 very recent double-blind, placebo- and mesalazine-controlled trials have shown that available 5-lipoxygenase inhibitors (zileuton and MK-591) were not significantly better than placebo in the maintenance of remission of ulcerative colitis.[88,89]

10.2 Topical Short-Chain Fatty Acids

Short-chain fatty acids (SCFA; acetate, propionate and *n*-butyrate) are organic acid products of the luminal anaerobic fermentation of carbohydrate and protein in the colon, with a strong and distinctive odour. They are essential for the nutrition and regeneration of epithelial cells. It has been suggested that colitis is associated with a defect in mitochondrial fatty acid oxidation with the resultant use of alternative pathways for fuel utilisation.[90]

Open, uncontrolled studies[91,92] have shown that a short cycle of rectal sodium butyrate 80 to 100 mmol/L appeared to be of some efficacy in the treatment of distal active ulcerative colitis. Moreover, combination therapy using enemas containing butyrate 80 to 100 mmol/L and mesalazine 1g administered twice daily for 4 weeks was effective in 5 patients with refractory distal ulcerative colitis.[93] However, 2 recent small, placebo-controlled trials provided conflicting data concerning the actual efficacy of SCFA in the treatment of this disease.

In a randomised, double-blind, placebo-controlled study, Vernia et al.[94] tested the efficacy of a 6-week course of topical SCFA (100ml, twice daily enemas of sodium acetate 80 mmol/L, sodium propionate 30 mmol/L and sodium butyrate 40 mmol/L) in 40 patients with mild to moderate distal ulcerative colitis. 14 patients on SCFA improved compared with 5 in the placebo group. In the SCFA-treated group all parameters signifi-

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cantly improved except the number of bowel motions, whereas no significant changes were recorded in the control group. A statistically significant difference between the 2 treatment regimens, however, was observed only for intestinal bleeding, urgency and the patient self-evaluation score. The authors concluded that topical SCFA is effective in the treatment of distal ulcerative colitis.

On the other hand, Steinhart et al.[95] did not find any statistically significant difference between nightly butyrate (60ml of an 80 mmol/L sodium butyrate solution) and placebo enemas in 38 patients with active disease. In particular, clinical improvement was noted in 7 of 19 (37%) butyrate-treated patients and 9 of 19 (47%) placebo-treated patients ($p = 0.51$). Clinical remission was achieved in 3 patients in each group (16%).

Differences in the mixture, concentrations, volumes and frequency of administration of rectal SCFA, as well as differences in the clinical, endoscopic and histological scores, may explain these conflicting results. Thus, whether rectal administration of SCFA has a specific therapeutic role, if any, in the treatment of distal ulcerative colitis remains to be established.

10.3 Nicotine

Epidemiological studies have suggested an association between nonsmoking, or the discontinuation of smoking, and ulcerative colitis.[96] Nicotine may act by affecting mucin, blood flow or immune defences.[97]

Small, uncontrolled studies have suggested the efficacy of transdermal nicotine in the improvement of symptoms and endoscopic appearance, as well as a possible corticosteroid-sparing effect.[98]

This relationship between nicotine and ulcerative colitis was more formally studied in a 6-week, randomised, double-blind study of transdermal nicotine versus placebo patches in patients with active ulcerative colitis.[99] 72 patients with active left-sided ulcerative colitis were randomised to receive transdermal nicotine patches ($n = 35$) or placebo ($n = 37$). Incremental doses of nicotine were given and most patients tolerated doses of 15 to 25 mg/24h. Nicotine was superior in inducing clinical improvement and reducing stool frequency, urgency and mucous, while adverse effects were minimal. Results in maintenance of remission have been less promising. The same group failed to demonstrate a maintenance effect from nicotine patches in an 80-patient, placebo-controlled trial.[100]

The efficacy and safety of liquid enemas of nicotine tartrate for mildly to moderately active left-sided ulcerative colitis unresponsive to first-line therapy were evaluated in a pilot study of 10 patients.[101] After 4 weeks, 3 of 10 (30%) discontinued therapy within 7 days because of an inability to retain the liquid enemas, while 5 of 7 (71%) showed clinical and sigmoidoscopic improvement. Transient and mild adverse events occurred in 4 of 10 patients; however, given the low or undetectable serum nicotine concentrations, these adverse events are not likely to be related to the nicotine enemas.

Despite these encouraging results with either transdermal or rectal preparations, the role of nicotine has yet to be established. Until further trials aimed at defining the exact use of nicotine in the treatment of ulcerative colitis have confirmed the potential advantage, if any, of nicotine therapy, its administration for patients with ulcerative colitis must be tempered by the numerous and significant health risks that accompany such a practice. At the moment, patients with refractory distal ulcerative colitis may represent a particular subgroup of candidates for nicotine treatment as an adjunctive drug to conventional therapy.

10.4 Local Anaesthetics

Gel formulations of local anaesthetics [lidocaine (lignocaine), ropivacaine] appear to modify inflammation in patients with ulcerative colitis, possibly by modulating the neural component of inflammation or by affecting the release of eicosanoid mediators.[85]

In an uncontrolled study,[102] 100 patients with ulcerative colitis ranging from proctitis to total colitis were treated topically with lidocaine gel 800

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mg/day. Most had an excellent response with 10% remission in patients with proctitis and 85% in patients with proctosigmoiditis. The duration of therapy ranged from 6 to 34 weeks. However, the recurrence rate was high. Promising results were also obtained with ropivacaine given rectally as a gel.[103]

10.5 Bismuth Subsalicylate Enema

Pilot studies and one randomised, double-blind, controlled study of 63 patients with distal ulcerative colitis have shown that bismuth-carbomer (a synthetic polyacrylate) 432 mg/day enemas are as effective as mesalazine enemas in the treatment of patients with distal active ulcerative colitis.[85] The proposed mechanisms of action may involve increasing the integrity of the mucus gel layer or interfering with bacterial adherence.

10.6 Sucralfate

Sucralfate is a cytoprotective mucopolysaccharide barrier agent used in the treatment of peptic ulcer disease. Although open studies have suggested that rectal sucralfate is an effective treatment for active distal ulcerative colitis, controlled trials against either mesalazine[104] or hydrocortisone enemas[105] have shown that this drug is of no therapeutic value for this disease.

10.7 Clonidine

Clonidine is a centrally acting α_2-agonist used predominantly as an antihypertensive agent. In a 30-week trial of 45 patients with active ulcerative colitis, daily divided doses of clonidine 0.9mg, prednisolone 60mg and sulfasalazine 4.5g were compared. Clonidine had similar efficacy to prednisolone in improving symptoms and sigmoidoscopic appearance. Its efficacy may be explained by the centrally mediated modulation of bowel motility.[106]

10.8 Free Radical Scavengers

Reactive oxygen metabolites (ROM) are partially reduced oxygen species that include superoxide, hydrogen peroxide, hydroxyl radical and hypohalous acids, playing a critical role in mediating cellular injury. Uncontrolled trials[85] have shown that superoxide dismutase, allopurinol and dimethylsulfoxide are effective in the treatment of active ulcerative colitis, and in maintaining remission when combined with sulfasalazine, producing relapse rates of only 5% at 12 months.

10.9 Heparin

The use of anticoagulant therapy in inflammatory bowel disease is tempered by the risk of generating uncontrolled bleeding. There is evidence that patients with active colitis have a hypercoagulable condition and increased risk of thromboembolic events. Gaffney et al.[107] reported remission in 9 of 10 patients with refractory ulcerative colitis receiving short term treatment with heparin (from 10 000 to 36 000 U/day). Remission was maintained with subcutaneous heparin 10 000 U/day during a 6-month follow-up period. These results were further confirmed by a very recent report of 16 cases of patients with corticosteroid-resistant ulcerative colitis.[108]

10.10 Chloroquine and Hydroxychloroquine

An abnormal process and presentation of antigen from intestinal epithelial cells to the mucosal immune system has been shown in inflammatory bowel disease. This abnormality may be reversed by the antimalarials chloroquine and hydroxychloroquine. These drugs have demonstrated some efficacy in the treatment of distal ulcerative colitis.[109,110]

11. Therapeutic Options for Different Forms of Distal Ulcerative Colitis

11.1 Mild to Moderate Activity

Patients with mild to moderately active disease (table I) are usually treated as outpatients. The therapeutic options include oral and/or topical salicylates or corticosteroids. The choice depends on a variety of factors, such as patient compliance and preference, cost and the formulations available.

Sulfasalazine 4 to 6 g/day in divided doses and given with meals has been a standard approach for patients without sulfonamide allergy. Alternatively, an oral mesalazine formulation may be prescribed, at divided doses of 2 to 4 g/day. These drugs are generally effective within 2 to 4 weeks and efficacious in 40 to 80% of patients. Rectal administration of corticosteroids or mesalazine is equally or more effective initial therapy than oral approaches for distal colitis, and by this route these drugs provide rapid relief of rectal symptoms. The choice of preparation will depend on the upper extent of the disease, with suppositories or foam reaching to a distance of about 15 to 20cm and enemas distributing the active drug to approximately the splenic flexure. The minimum dosage of mesalazine enemas or foam is 1 g/day for a period of 4 to 8 weeks. However, several regimens may be used, and the dosage may be increased up to 2g twice daily. If suppositories are chosen, the mean daily dosage is 500mg twice daily.

The dosage of topical corticosteroids depends on the type of drug available and whether it is an older or newer topical corticosteroid. Traditional rectal corticosteroids (hydrocortisone, prednisolone, betamethasone) can be used alone in exacerbations of distal disease. Moreover, they can be used in conjunction with oral salicylates. Suppositories and foam enemas can be given at any time of day. Liquid enemas are best administered once daily, in bed. These formulations are generally efficacious within 2 to 4 weeks and are effective in about two-thirds of patients. Also, non-systemically active corticosteroids in enema form, especially budesonide 2 mg/day and beclomethasone 2, 3 and, rarely, 6 mg/day, are at least as effective as, if not more so than, the standard rectal corticosteroids.

Patients presenting with moderately severe disease benefit from a short cycle of systemic corticosteroids (for example, prednisone or methylprednisolone 20 mg/day), associated with topical mesalazine or corticosteroids.

11.2 Severe Activity

Ulcerative colitis can present as a medical emergency when severe, fulminating or even as a toxic megacolon. The definitions between these categories are blurred because of the range and diversity of presentation and extent of disease. Even patients with left-sided colitis, although less frequently than those with universal colitis, may develop fulminating disease or toxic megacolon. In all these cases the patients must be hospitalised immediately. The treatment recommended is an intensive medical regimen similar to that described by the Oxford group[111] and further applied by Järnerot et al.[112] This includes:

- nutritional parenteral therapy, with fluid and electrolyte replacement;
- parenteral corticosteroids (for example, methylprednisolone 40 to 60 mg/day, or hydrocortisone 200 to 300 mg/day);
- topical mesalazine or corticosteroids;
- broad-spectrum antibacterials (for example, metronidazole, gentamicin, piperacillin or cephalosporin)

Profound anaemia requires transfusion to maintain a packed cell volume greater than 30% in patients with active haemorrhage. Any such patient should be evaluated by both a gastroenterologist and a surgeon. This intensive treatment must be continued for 5 to 10 days. If after this period the clinical condition of the patient is unchanged or worsened, intravenous treatment with cyclosporin must be started, at a dosage of 4 mg/kg/day, for at least 4 to 7 days before colectomy. The use of this potentially toxic drug must be managed by expert clinicians at the referral tertiary centre.

11.3 Refractory Distal Ulcerative Colitis

Patients with distal ulcerative colitis not responding within 6 to 8 weeks to oral treatment with sulfasalazine, or one of the mesalazine formulations combined with topical corticosteroids, or mesalazine enemas, are defined as being refractory to conventional therapy.[113] However, before patients are placed in this subgroup, treatment failure

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due to factors such as drug intolerance or failure to retain therapeutic enemas must be excluded. Moreover, conditions such as infectious diseases, rectal prolapse, solitary ulcer syndrome or radiation proctitis must be considered in the differential diagnosis.

Patients with this form of distal ulcerative colitis are recommended to maintain the oral treatment but to change topical treatment from corticosteroids to mesalazine for a period of 4 to weeks. If they are still symptomatic, systemic corticosteroids in a dosage corresponding to prednisolone 40 mg/day should be added, for a period of not more than 8 to 10 weeks. Patients refractory to treatment with oral sulfasalazine or another mesalazine-based drug plus topical or systemic mesalazine should be treated with topical or systemic corticosteroids. In this context, topically acting corticosteroids, especially budesonide, may play a therapeutic role.

Other therapeutic options must be considered for patients who do not respond to these treatments, such as immunosuppressive therapy (azathioprine or mercaptopurine), combination of mesalazine and topically active corticosteroid enemas, local anaesthetics, nicotine and SCFA.

Another possible therapeutic tool in the treatment of distal refractory disease is acetarsol, an organic arsenical, used as a suppository containing 0.25g of the drug, widely used in Northern Europe. In a 3-week double-blind trial,[114] acetarsol was compared with prednisolone-21-phosphate 5mg. Efficacy was similar with both drugs, with a trend to greater benefit in the acetarsol group. Further studies, however, are necessary to elucidate the therapeutic role of this drug in the treatment of distal ulcerative colitis.

11.4 Maintenance Therapy

The clinical pattern of ulcerative colitis (intermittent chronic, chronically active and frequently relapsing disease) and patient compliance and preference could be two useful guidelines as to the choice of drug and the route of administration for the long term treatment of ulcerative colitis in remission. Sulfasalazine and mesalazine[115,116] are the first-choice drugs for the maintenance treatment of patients who have chronic intermittent disease. The standard dosage of sulfasalazine is 2 g/day,[117] while the optimum dosage of oral mesalazine is controversial.

Indeed, a trend towards a higher remission rate was noted with higher doses of mesalazine, especially in patients with a minor duration of remission before entering the study.[118-120] In particular, olsalazine was most effective at a higher dosage (2 g/day) in patients with proctitis (90% remission on 2 g/day, $p = 0.03$) or in those in remission for less than 12 months before the trial entry (88% remission on 2 g/day, $p = 0.0006$).[118]

The use of mesalazine suppositories (500mg twice daily)[121,122] or mesalazine enemas, at different dosages and dosage regimens (daily, nightly or every other day, intermittently),[65,123] may represent an alternative therapeutic approach to maintaining remission in patients with distal ulcerative colitis.

For patients with chronically active or corticosteroid-dependent ulcerative colitis who have achieved full remission while taking azathioprine or mercaptopurine, the maintenance treatment consists of prolonging this immunosuppressive therapy for at least 2 years.[72-74] Preliminary data from open, uncontrolled studies suggest that similar therapy seems to be effective in patients with severe ulcerative colitis who achieved remission with intravenous cyclosporin treatment.[78,79]

11.5 Surgery

The indications for surgical treatment in patients with distal ulcerative colitis, especially with left-sided colitis, are very limited.

Patients with chronically active disease, resulting in physical debility and psychosocial dysfunction with very low quality of life, who are refractory to standard medical treatment, and not achieving remission after immunosuppressive treatment with azathioprine or mercaptopurine because of their ineffectiveness or toxicity, are candidates for surgical treatment.

Moreover, although rarely, patients with severe left-sided ulcerative colitis, unresponsive to maximal intravenous medical therapy (cyclosporin included) are also best served by colectomy.

12. Conclusions

As the aetiology of inflammatory bowel disease is still unknown, the major objective of drug therapy is to improve the quality of life by rapidly suppressing the symptoms of inflammation, without introducing major adverse effects.

Conventional corticosteroids, systemically and topically administered, are effective in reducing the inflammation in about two-thirds of patients with active distal ulcerative colitis, but they should not be used for maintaining remission. Considering that prolonging treatment with classic glucocorticosteroids includes the risk of severe systemic adverse effects, enemas with topically acting corticosteroids are likely to replace conventional corticosteroid enemas in the near future, especially in those patients who need long term or high dose therapy. Trials of oral formulations with colonic controlled release of these topical corticosteroids are warranted.

Moreover, it seems necessary to clarify the advantage of a limitation of oral as opposed to topical corticosteroids when a short course with higher dosage is indicated and which is also probably more effective and more likely to eliminate mucosal inflammation.

Sulfasalazine and oral mesalazine are therapeutically effective in the treatment of distal ulcerative colitis, both active and quiescent. However, no significant difference exists between these 2 drugs and among the various mesalazine formulations.

The efficacy, safety and acceptance of rectal formulations of mesalazine in the short term treatment of active distal ulcerative colitis are well established. Thus, in this form it may be the initial treatment of choice when the disease is limited to the distal colon. Well designed additional trials are needed to confirm the efficacy of rectal mesalazine, alone or in association with oral therapy, in maintaining disease remission.

Combination therapy of a topical corticosteroi with a mesalazine enema or with mesalazine table and enemas is a promising treatment for patien with active distal ulcerative colitis, and its potenti in refractory disease in particular seems promisin Further investigation is required to better define i exact therapeutic role.

For patients who have a disease which flares u when corticosteroids are reduced, as well as i those with chronically active disease refractory t conventional treatment, azathioprine and mercap topurine are effective and well tolerated in avoic ing colectomy and in reducing both corticosteroi requirements and clinical relapses. These 2 agen have also shown their effectiveness in maintainin remission of ulcerative colitis. Cyclosporin is e fective in inducing remission in patients wit acutely severe disease who do not achieve remi sion with intensive standard therapy.

Azathioprine and mercaptopurine seem to be e fective in prolonging remission in this group of pa tients. The role of both oral and systemic metho trexate in the treatment of distal ulcerative coliti awaits clarification.

5-Lipoxygenase inhibitors, rectal SCFA, nicc tine, local anaesthetics, bismuth subsalicylate er ema, clonidine, free radical scavengers, hepari and hydroxychloroquine are alternative drugs ur der investigation and the future will show if the will be given a place in the treatment of distal ul cerative colitis.

Patients with chronically active disease, espe cially with left-sided colitis, unresponsive either t medical treatment or immunosuppressive therap (azathioprine or mercaptopurine), are candidate for surgical treatment.

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Effect of Cyclosporine in a Murine Model of Experimental Colitis

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The use of immunosuppressive therapy may be associated with significant toxicity. The aim of this study was to investigate the effect of cyclosporine A (CsA) in murine model of experimental colitis. Experimental colitis was induced in NMRI mice using an enema of 0.2% solution of dinitrofluorobenzene, combined with skin sensitization. After inducing colitis, experimental groups of animals were treated with CsA (1, 3, 5, 10, 25, 50 mg/kg/day) intraperitoneally (i.p.) or intracolonically (i.c.), and control groups were treated with phosphate-buffered saline intraperitoneally or intracolonically, respectively. Colonic inflammatory changes were assessed using a histopathologic score of 0–30, and pooled whole blood samples were processed with monoclonal antibodies for cyclosporine concentration. In addition, two groups of animals with experimental colitis were treated intraperitoneally or intracolonically with 3 mg/kg/day of CsA, and the colons were also taken for immunohistochemistry for CD25. CsA diminished the extent of colitis in groups treated with 3, 5, 10, or 25 mg/kg intraperitoneally or intracolonically, and in groups treated with 1 and 50 mg/kg intracolonically ($P < 0.05$). The effect of intracolonic application of CsA was not related to whole blood cyclosporine concentrations. In addition, the effect of CsA at 3 mg/kg, applied intraperitoneally or intracolonically was, in part, expressed in decreasing the numbers of CD25+ cells within colonic mucosa/submucosa ($P < 0.05$). In conclusions, the results of this study indicate the possibility of intracolonic application of cyclosporine in order to widen the therapeutic window for effective, but possibly toxic drug, such as cyclosporine.

KEY WORDS: inflammatory bowel disease; experimental colitis; inflammation; cyclosporine A.

The treatment for patients with active ulcerative colitis (UC) and active Crohn's disease (CD), collectively referred to as inflammatory bowel disease (IBD), includes immunosuppressive and immunomodulating agents such as azathioprine/6-mercaptopurine, methotrexate, tacrolimus, and cyclosporine A (CsA). Recent studies documented the effect of infliximab, a chimeric monoclonal antibody targeting tumor necrosis factor (TNF-α), in inducing remission in active CD and healing CD-related fistulas (1). Thalidomide has previously been shown to be effective in inflammatory disorders in which TNF has been implicated as an important mediator in inflammation, and two recent open-labeled clinical trials have shown its efficacy in refractory CD, even in patients in whom therapy with CsA and infliximab has failed (2, 3).

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Despite an increasing number of treatment options, however, many patients with active IBD experience serious side effects from newly designed medical interventions, including therapy with infliximab and thalidomide in patients with CD. The narrow therapeutic window and documented potential toxicity have, currently, confined the use of intravenous therapy with CsA to clinical trials in medical centers and for treatment of severe, refractory disease when surgery is not appropriate or before other therapies have taken effect (4, 5).

The aim of this study was to investigate the effect of CsA, given intraperitoneally or intracolonically in a form of an enema, on acute inflammatory lesions in a hapten-induced murine model of experimental colitis.

MATERIALS AND METHODS

Animals. NMRI male mice, 10 to 12-week old (30–35 g) were used in all experiments. All experimental and control groups consisted of six to eight animals in each group. They were fed pelleted diet and supplied with drinking water *ad libitum*.

Induction of Colitis. Animals in both experimental and control groups were pretreated with two sensitizing doses of 0.1 ml of 0.5% 2,4-dinitrofluorobenzene (DNFB) by rubbing the substance on previously shaved abdominal skin, 96 and 72 hr before challenge. The DNFB solution was prepared by diluting the original DNFB preparation (Sigma Chemical Co., St. Louis, Missouri, USA) with 4:1 acetone–olive oil. Freshly prepared solutions were used for each application. Experimental colitis was induced in all animals by receiving the challenge enema of 0.025 ml of 0.2% DNFB solution in acetone and olive oil. Enemas were administered under light ether anesthesia, by using a 4-cm plastic catheter with an outer diameter of 0.9 mm, attached to a glass syringe, as previously described (6, 7).

Application of Cyclosporine. In first part of the study, 12 experimental groups of animals (N = 6–8/per group) were treated with different doses of CsA (1, 3, 5, 10, 25, or 50 mg/kg/day) given intraperitoneally or in a form of an enema intracolonically, starting 6 hr after induction of colitis with DNFB and for consecutive five days. Two control groups were treated with an equivolume of phosphate buffered saline (PBS) intraperitoneally or intracolonically, in the same manner after induction of colitis with DNFB.

In second part of the study, two experimental groups of animals (N = 6–8) were treated with same dose of CsA (3 mg/kg/day) given intraperitoneally or intracolonically, 6 hr after induction of colitis and for consecutive five days. One control group was treated with PBS intracolonically, in the same manner after induction of colitis. The second control group consisted of untreated healthy animals.

Different doses of CsA (1, 3, 5, 10, 25, or 50 mg/kg) were applied intraperitoneally or in a form of an enema in an equalvolume of 0.1 ml, in one daily dose and at the same time each day. Different doses of CsA were obtained from the original preparation for parenteral use (Sandimmun, Sandoz, Basel, Switzerland) by diluting the original substance with phosphate-buffered saline. For intraperitoneal application of CsA, we used a small needle, attached to a syringe. CsA enema was applied using a 4-cm-long plastic catheter with an outer diameter 0.9 mm and attached to a syringe.

TABLE 1. HISTOLOGIC SCORE DENOTING THE TOTAL OF PARTICULAR SCORES FOR EACH OBSERVED PARAMETER OF INFLAMMATION IN MICE COLON

Ulceration and necrosis	0–3
Oedema	0–2
Infiltration with PMNs surrounding ulcers and necroses	0–3
Infiltration with PMNs surrounding blood vessels	0–3
Infiltration of lamina propria with mononuclear cells	0–3
Infiltration of submucosa with mononuclear cells	0–3
Loss of integrity of lamina muscularis mucosae	0–5
Inflammatory changes of colonic serosa	0–5
Thickness of colonic wall	0–3
TOTAL	0–30

Harvesting. In first part of the study, all the animals were killed on day 5 after induction of colitis with DNFB. The entire colon of each animal was longitudinally opened and rolled up in a reel with the mucosal surface on the outside. The tissue was fixed in buffered formalin and stained with hematoxylin and eosin.

In addition, the pooled whole blood samples of each group of animals were processed for cyclosporine concentration using the fluorescent polarization immunoassay (FPIA, Abbott) with monoclonal antibodies (Abbott Laboratories) (8).

In second part of this study, all the animals were killed on day 5 after induction of experimental colitis, including the group of untreated healthy animals. The entire colon of each animal was longitudinally dissected in two halves. One half was taken for histology (HE) and processed in the manner described previously. The other half was also rolled up in a roll, and frozen in acetone and CO_2, and stored at −70 °C. Sections were cut on a cryostat and processed with monoclonal antibodies for IL-2R/CD25 (anti-mouse IL-2R/CD25, Boehringer Biochemica, Mannheim, Germany). The same sections were then processed with secondary antibodies (anti-rat peroxidase conjugate; Sigma) and stained (DAB: 3,3-diaminobenzidine; Sigma) (9).

Histology. The cross-sections of colonic tissue rolls were analyzed by a trained pathologist unaware of treatment regimens. A modified scoring scale, based on the presence of different parameters of inflammatory tissue injury was developed according to previously published studies (10, 11). The inflammatory score range is 0–30 and denoting the total of particular scores for each observed inflammation parameter (Table 1). Score 0 represents the histology feature of the colon of healthy untreated mice. Score 30 represents the histology feature of the most severe inflammatory lesions in the control group of animals with experimental colitis, treated with PBS (Figure 1). Data were analyzed using nonparametric Kruskal-Wallis and Mann-Whitney statistical methods. The extent of observed lesions was expressed as mean score with SD. Mean score was practically identical to the median for each group of animals. Results were considered significant at $P < 0.05$ (7, 12).

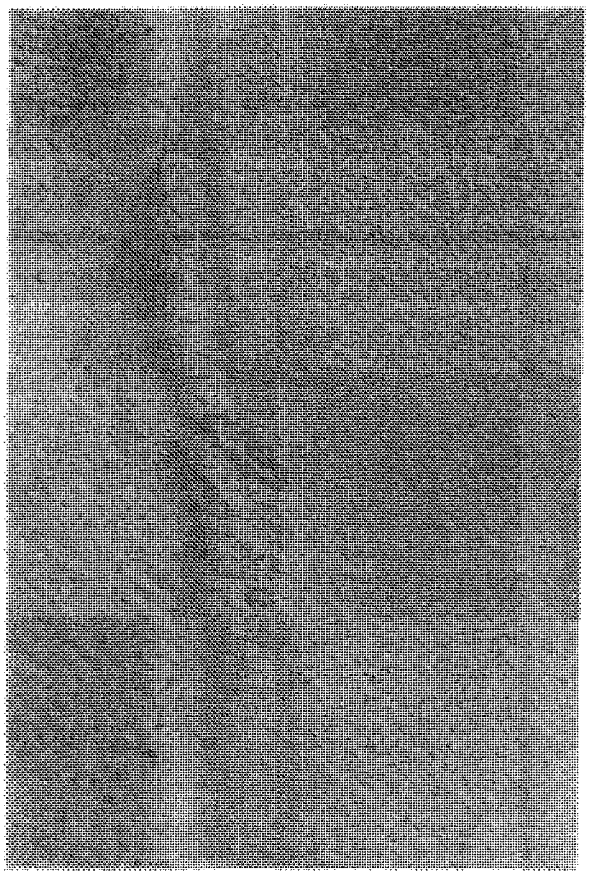

Fig 1. The edge of necrosis in the colon of mouse from control group treated with phosphate buffered saline intracolonically, after induction od experimental colitis. Note the enhanced wall thickness, loss of normal mucosal and submucosal architecture, and expressed infiltration with inflammatory cells (HE, 10×10)

Immunohistochemistry. The distribution density of immunoreactive cells for CD25 in mucosa/submucosa of mice colon was measured by counting cell numbers using a photometric glide in the light microscope (Ortholux 6.3 × 1, 25 × 40). The specimens used for this analysis showed neither macroscopic ulcerations nor fibrotic lesions. The data of each specimen were presented by mean number in 20 different areas from two to three cross-section slides. All data were presented as mean ± SD in each group. These values were compared using Kruskal-Wallis test and considered significant at $P < 0.05$ (13). For the purpose of statistical analysis we used software (CSS: Complete Statistical System, Stat Soft Inc.).

RESULTS

In the first part of the study, we observed the effect of various doses of CsA (1, 3, 5, 10, 25, 50 mg/kg) applied intraperitoneally (i.p.) or in a form of an enema intracolonically on inflammatory lesions in experimental colitis induced with DNFB (Figure 2 and 3). CsA was effective in diminishing the extent of provoked colonic inflammation in experimental groups treated with doses of 3, 5, 10, or 25 mg/kg i.p. or i.c., as well as in groups treated with 1 mg/kg and 50 mg/kg i.c. ($p < 0.05$). In groups treated with 1 mg/kg CsA and 50 mg/kg CsA intraperitoneally there was no difference when compared to control groups with maximal observed lesions (Figure 2). Experimental groups treated with equal doses of CsA that proved to be effective (3, 5, 10, and 25 mg/kg) did not differ in effect significantly in regard to the method of application intraperitoneally or intracolonically (data not shown).

In all experimental groups treated with CsA intraperitoneally, we documented the presence of cyclosporine in pooled whole blood samples of each group of animals (Table 2). The lowest concentration was

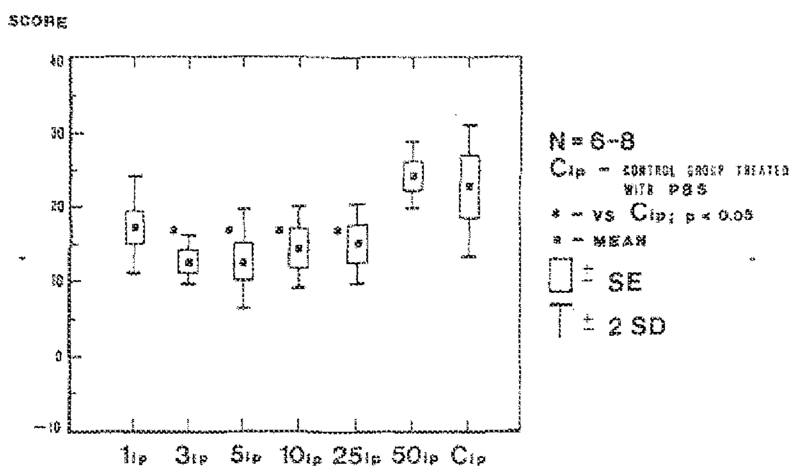

Fig 2. The effect of 1, 3, 5, 10, 25, and 50 mg/kg/day of cyclosporine A applied intraperiotneally on inflammatory lesions in hapten-induced experimental colitis in mice.

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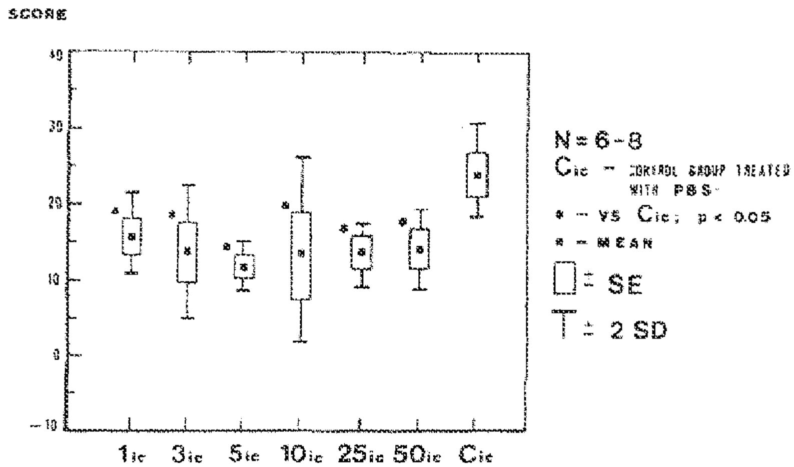

Fig 3. The effect of 1, 3, 5, 10, 25, and 50 mg/kg/day of cyclosporine A applied intracolonically on inflammatory lesions in hapten-induced experimental colitis in mice.

noted in the experimental group treated with 1 mg/kg intraperitoneally (122 ng/ml) and the highest in experimental group treated with 50 mg/kg CsA intraperitoneally (>1500 ng/ml). In experimental groups treated with CsA in a form of an enema intracolonically, we documented the presence of cyclosporine in pooled whole blood samples in groups treated with 10, 25, or 50 mg/kg intracolonically, and the observed concentrations were lower than in the groups treated with equal doses of CsA intraperitoneally. In groups treated with 1, 3, or 5 mg/kg of CsA intracolonically, the presence of cyclosporine in whole blood samples was not detectable.

In the second part of the study, we observed the effect of an equal dose of CsA (3 mg/kg) applied intraperitoneally or intracolonically on inflammatory lesions in experimental colitis and the number of CD25+ inflammatory cells within colonic mucosa as well. CsA was effective in diminishing the histologic extent of provoked colonic inflammation in both experimental groups treated with 3 mg/kg, i.p. or i.c. ($p < 0.05$) (Figure 4). Induction of experimental colitis with DNFB significantly increased the number

TABLE 2. CONCENTRATION OF CYCLOSPORINE (NG/ML) IN WHOLE BLOOD SAMPLES FROM EXPERIMENTAL GROUPS OF ANIMALS TREATED WITH DIFFERENT DOSES OF CYCLOSPORINE INTRAPERITONEALLY OR INTRACOLONICALLY

	Concentration of CsA (ng/ml., FPIA/Mab-S)*					
Dose (mg/kg)	1	3	5	10	25	50
i.p.	122	369	556	635	1347	>1500
i.c.	—	—	—	125	211	240

*FPIA/Mab-S: fluorescent immunoassay with monoclonal antibodies (TDX/FLX Abbot Laboratories, USA).

of CD25+ inflammatory cells within colonic mucosa when compared to colonic mucosa of healthy, untreated animals ($p < 0.05$) (Figure 5). CsA in a dose of 3 mg/kg, applied intraperitoneally or intracolonically significantly decreased the number of CD25+ inflammatory cells, when compared to control group with experimental colitis treated with PBS intracolonically ($p < 0.05$) (Figure 5). There was no difference between experimental groups in regard to the way of application of CsA.

DISCUSSION

Experimental models of IBD in animals allow the study of various components of intestinal inflammation and enable the development of novel therapeutic modalities, in ways that are not possible in humans. However, the results obtained in experimental models can complement and expand studies in humans but do not replace them (14). Experimental models of intestinal inflammation generated by methods of genetic engineering in transgenic rats or mutant mice have recently become available and allow the study of early factors of intestinal inflammation, cascade of proinflammatory/antiinflammatory cytokines, and particularly the complex interplay between the genetic background, immune system, and environment (14, 15). On the other hand, models that have been available for some time, including models of experimental colitis induced with hapten molecules, allow the study of nonspecific inflammation and wound healing (15).

Experimental colitis induced with hapten molecules, such as trinitrobenzene sulfonic acid (TNBS)

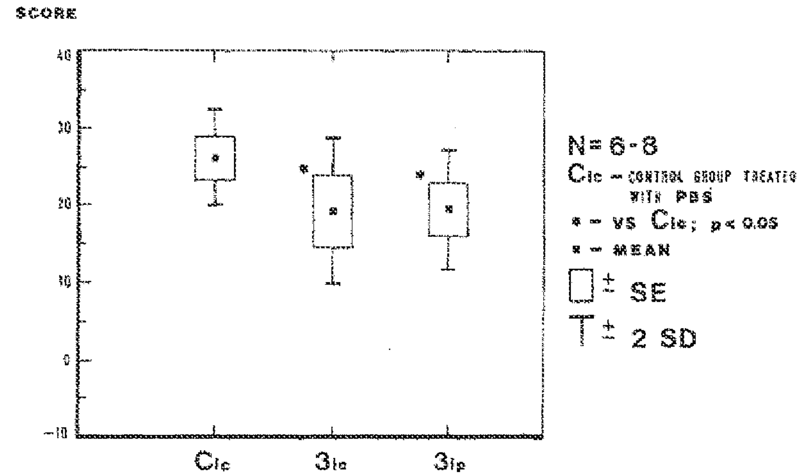

Fig 4. The effect of 3 mg/kg/day of cyclosporine A applied intracolonically or intraperitoneally on inflammatory lesions in hapten-induced experimental colitis in mice.

and 2,4-dinitrifluorobenzene (DNFB), results in acute necrosis after the challenge enema, usually transmural, due to oxidative damage. Acute necrosis and inflammation in the first three to five days after the challenge enema are followed by chronic inflammation with a mononuclear infiltrate, mainly in the submucosa, that lasts a variable amount of time (6, 7). In mice, a hapten-induced colitis (including DNFB-induced experimental colitis) appears to be a classic (DTH) (delayed, type hypersensitivity) response mediated by T cells responding to hapten-modified self-antigen. This results in an increased production of IFN-gamma and IL-2 by CD4+ T cells that is consistent with the Th-1 effector response and involves increased production of IL-12 by non-T cells such as macrophages and/or dendritic cells (16). The characteristic histologic pattern of provoked colonic injury in DNFB-induced experimental colitis models, and availability of a quantitative scoring system of pathology representing a simple, inexpensive, and reasonably reproducible well defined system, allowed the study of the documented antiinflammatory effect of corticosteroids in this particular model of Th-1-driven intestinal inflammation (6, 7, 10, 11, 17–19).

The results of this study lead to several conclusions in regard to the effect of CsA on acute inflammatory lesions in Th-1-mediated experimental colitis. First, it was possible to document the effect of CsA, applied intraperitoneally and intracolonically, starting the treatment 6 hr after the challenge enema with DNFB.

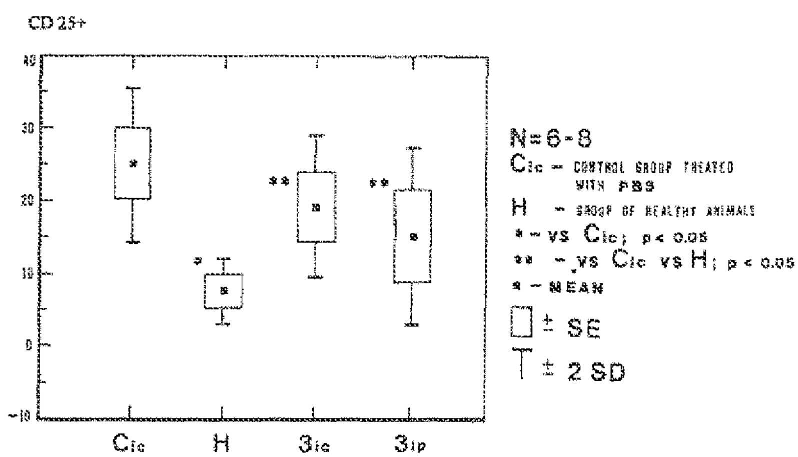

Fig 5. The effect of 3 mg/kg/day of cyclosporine A applied intracolonically or intraperitoneally on number of CD25⁻ cells in colon of mice with hapten-induced experimental colitis.

In most published studies, CsA exerted the maximal effect in suppressing the DTH reaction when applied before the activation stimulus (20). Thus, it could be expected that the effect of CsA would be diminished or lacking in the situation of established inflammation. It is possible that the observed antiinflammatory effect of CsA was mediated by neutralization of chemotactic properties of cyclophylin on neutrophils and eosinophils, whereas the specific immunomodulatory effect of CsA and inhibition of cytokine secretion were playing a minor role in established inflammation. Furthermore, it has been shown *in vitro* that CsA is not capable of inhibiting the secretion of IL-2 by human lamina propria T cells in response to CD2 activation (21). This could also, in part, explain the difference in effect of CsA on changes in Crohn's disease and ulcerative colitis in humans (22–24).

Second, the effect of CsA applied intraperitoneally was in part dependent on the concentration of cyclosporine obtained in pooled whole blood samples of experimental groups of animals. The lack of an antiinflammatory effect in the experimental group treated with 1 mg/kg CsA intraperitoneally (obtained cyclosporine blood concentration 122 ng/ml) is possibly due to the suboptimal presence of the drug in blood, as well as in intestinal mucosa. On the other hand, the lack of antiinflammatory effect in the experimental group of animals treated with 50 mg/kg CsA (obtained cyclosporine concentration >1500 ng/ml) could possibly be explained by potential adverse effect in mice. It is well documented that CsA has no direct toxic effect on intestinal mucosa and it seems possible that, excessive dose of CsA could modulate the central (thymus-dependent) and also peripheral (in tissues) mechanisms of tolerance to "self" antigens (25–27).

Third, the effect of CsA, applied intracolonically, was not influenced by its concentration, and the antiinflammatory effect was noted as significant in experimental groups with no detectable cyclosporine in pooled whole blood samples. The presence of cyclosporine in pooled whole blood samples of experimental groups treated with 10, 25, or 50 mg/kg of CsA intracolonically could be due to an enhanced permeability of epithelial barrier and resorption through the inflamed mucosa. The results of published studies indicate that the application of cyclosporine in the form of an enema resulted with low systemic bioavailability and drug concentration within the intestinal mucosa, significantly higher than the concentration obtained in whole blood samples of the same individuals (28). These observations could also in part explain the significant effect of CsA in experimental groups treated with 1 and 50 mg/kg of CsA intracolonically, and the lack of antiinflammatory effect in experimental groups treated with same doses of CsA intraperitoneally.

In light of this, we find it important to stress that in groups treated with 3, 5, 10, or 25 mg/kg CsA, the significant antiinflammatory effect of CsA in this study was influenced neither by given the dose, the method of application, the cyclosporine concentrations in pooled whole blood samples.

Induction of experimental colitis with DNFB also resulted in a significant increase in the number of CD25+ inflammatory cells within the colonic mucosa. This is consistent with previously the published observation of enhanced IL-2 activity in experimental colitis induced with TNBS (29). In our study, we noted the significant effect of 3 mg/kg CsA, applied intraperitoneally or intracolonically in decreasing the number of CD25+ inflammatory cells within the colonic mucosa, partly as a result of specific mechanisms such as blockade of IL-2, TNF-α, and IFN-γ synthesis and secretion by lamina propria T cells. However, it seems reasonable to assume that the effect, in this setting, was also expressed on less specific inflammatory components by direct or indirect interaction and influence on dysfunction/permeability of epithelial barrier and infiltration with polymorphonuclear leukocytes (18). The balance of these two modes of action seems to be dependent on dose, method, and time of application of CsA.

Thus, in this particular model of Th-1-mediated experimental colitis, we documented the possibility of widening the therapeutic window for an effective, but possibly toxic drug, such as CsA. In clinical setting, the average oral dose of 8 mg/kg/day CsA has a modest therapeutic gain of 27% in patients with active CD and continuous intravenous infusion of CsA (4 mg/kg/day), for up to several weeks represents an experimental option with no standard indication for patients with fistulizing and severe inflammatory CD (30). However, the results of this experimental study could imply the possibility of intracolonic CsA application as a part of immunosuppressive therapy, maybe in a non sustained (sequential) combination with other immunosuppressive drugs in patients with severe inflammatory CD of the colon. Generally speaking, intracolonic application of candidate drugs and substances with antiinflammatory and immunomodulatory properties seems to offer a new way of delivering therapeutic modalities, including the gene

therapy, targeted to suppression of intestinal inflammation.

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Vernal keratoconjunctivitis

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REVIEW

Abstract

Vernal keratoconjunctivitis (VKC) is an allergic eye disease that especially affects young boys. The most common symptoms are itching, photophobia, burning, and tearing. The most common signs are giant papillae, superficial keratitis, and conjunctival hyperaemia.

Patients with VKC frequently have a family or medical history of atopic diseases, such as asthma, rhinitis, and eczema. However, VKC is not associated with a positive skin test or RAST in 42–47% of patients, confirming that it is not solely an IgE-mediated disease. On the basis of challenge studies as well as immunohistochemical and mediator studies, a Th2-driven mechanism with the involvement of mast cells, eosinophils, and lymphocytes has been suggested. Th2 lymphocytes are responsible for both hyperproduction of IgE (interleukin 4, IL-4) and for differentiation and activation of mast cells (IL-3) and eosinophils (IL-5). Other studies have demonstrated the involvement of neural factors such as substance P and NGF in the pathogenesis of VKC, and the overexpression of oestrogen and progesterone receptors in the conjunctiva of VKC patients has introduced the possible involvement of sex hormones. Thus, the pathogenesis of VKC is probably multifactorial, with the interaction of the immune, nervous, and endocrine systems.

The clinical management of VKC requires a swift diagnosis, correct therapy, and evaluation of the prognosis. The diagnosis is generally based on the signs and symptoms of the disease, but in difficult cases can be aided by conjunctival scraping, demonstrating the presence of infiltrating eosinophils. Therapeutic options are many, in most cases topical, and should be chosen on the basis of the severity of the disease. The most effective drugs, steroids, should however be carefully administered, and only for brief periods, to avoid secondary development of glaucoma.

A 2% solution of cyclosporine in olive oil or in castor oil should be considered as an alternative. The long-term prognosis of patients is generally good; however 6% of patients develop corneal damage, cataract, or glaucoma.
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Keywords: allergic conjunctivitis; vernal keratoconjunctivitis; pathogenesis; treatment

Introduction

Vernal keratoconjunctivitis (VKC) is a member of a group of diseases classified as allergic conjunctivites including perennial and seasonal rhinoconjunctivitis, atopic keratoconjunctivitis, and giant papillary conjunctivitis.[1–3] For many years, all allergic conjunctivites were considered (as suggested by Coombs and Gell[4]) the expression of a classical type I IgE-mediated hypersensitivity reaction at the conjunctival level. More recent clinical observations, however, suggest that other tissues of the eye are also involved in the ocular allergic reaction: the lids, with their high content of mast cells, the tear film, with its immunoglobulins, and the cornea, so important for visual function. New discoveries regarding the pathogenesis of ocular allergies have clearly indicated that the participation of the entire ocular surface in allergic diseases is not only the consequence of tissue contiguity but derives from a complex exchange of information between these tissues through cell-to-cell communications, chemical mediators, cytokines, and adhesion molecules. It is also possible that the neural and endocrine systems may influence the ocular allergic response.

The purpose of this paper is to describe the clinical expression of VKC, to discuss its pathogenetic mechanisms, and to suggest novel therapeutic strategies.

Clinical features

VKC is a chronic bilateral inflammation of the conjunctiva characterized by hyperaemia, chemosis, photophobia, and filamentous and

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sticky mucous discharge. The hallmark of the disease is the presence of giant papillae (cobblestone appearance) at the upper tarsal conjunctiva (tarsal form) or at limbus (bulbar form).[1-3] Although there are some differences between the two forms, probably related to geographical variations, it seems likely that both represent different clinical manifestations of the same disease[3] (Figures 1–3).

VKC mainly affects boys in their first decade of life and the sequelae of the disease may be responsible for permanent visual impairment. Despite its name, the disease can be frequently present all year round. Approximately 23% of patients have a perennial form of VKC from disease onset and more than 60% have additional recurrences during the winter.[5] Furthermore, in almost 16% of the cases, the seasonal (vernal) form evolves into a chronic, perennial inflammation after a mean of 3 years from disease onset, suggesting that the longer patients suffer from VKC, the more apt they are to develop a persistent form of the disease.[5]

Itching, photophobia, burning, and tearing are the major ocular symptoms. Patients also complained of frequent conjunctival redness after exposure to nonspecific stimuli. This finding supports previous reports suggesting the presence of a conjunctival hyper-reactivity when sun, dust, wind, and other general climactic factors or nonspecific stimuli come in contact with the conjunctival mucosa.[6] This hyper-reactivity, which is also known to be frequently associated with asthma and other allergic diseases, may actually be a distinct clinical entity. At present, the exact mechanism for nonspecific hyper-reactivity is not understood, but it is possible that the release of vasoactive mediators could be involved.[7]

Signs include the presence of giant papillae on the upper tarsal conjunctiva or at the limbus, the presence of aggregates of epithelial cells and eosinophils at the

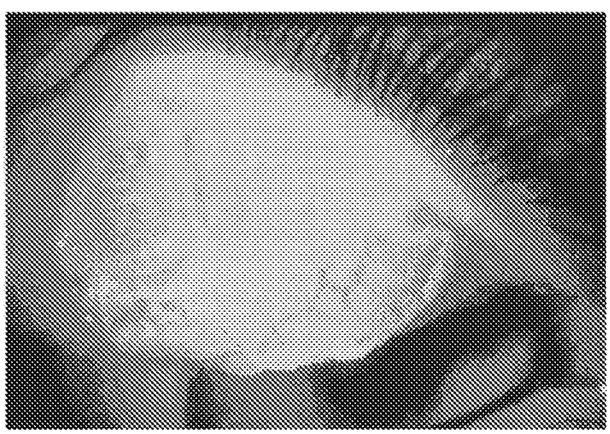

Figure 1 Giant papillary hypertrophy with the typical cobblestone appearance of the upper tarsal conjunctiva.

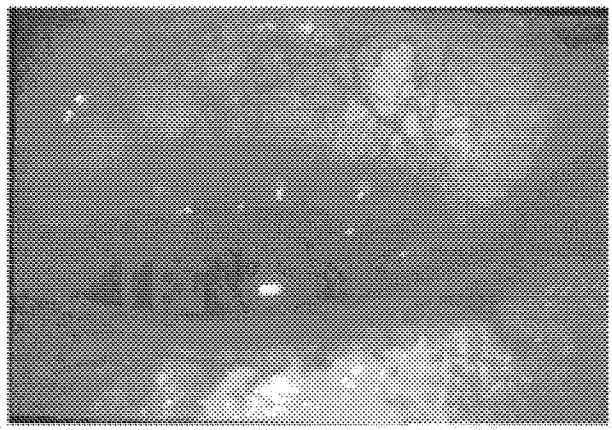

Figure 2 Trantas' dots in the superior limbus represent an aggregation of epithelial cells and eosinophils.

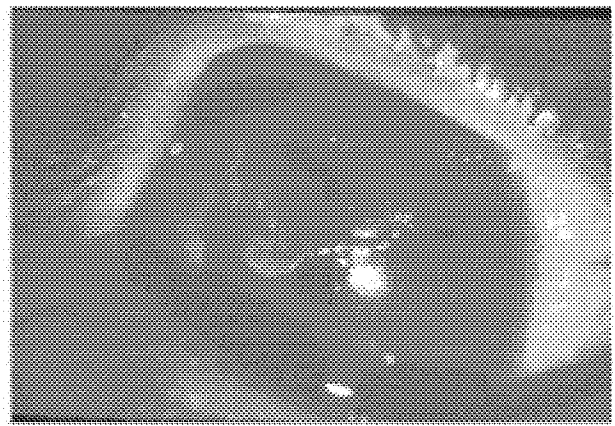

Figure 3 Corneal shield ulcers (Togby's ulcers) are mainly located in the upper paracentral cornea.

limbus (Trantas' dots), and marked conjunctival hyperaemia. Subconjunctival fibrosis, symblepharon, and conjunctival keratinization can develop. The cornea is almost always damaged with a superficial keratopathy, or the presence of corneal shield ulcers (occurring in approximately 3–11%) and neovascularization.[5,8,9] A blepharitis is frequently associated and eczema or maceration of the lid can be observed. Cataract and steroid-induced glaucoma are the major ocular complications.[5,9]

Immunologic mechanisms

Patients with VKC have a family history of atopic diseases in 49% of cases. These patients may also have a medical history of other atopic conditions including asthma (26.7%), rhinitis (20%), and eczema (9.7%).[5] Data regarding the frequency of sensitisation are conflicting: Ballow and Mendelson[10] reported 19% of positive

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sensitisation, Easty *et al*[15] reported 80%. In a large series of 195 patients, we found a positive response to skin tests and RAST in 57 and 52%, respectively.[5] Total IgE has been shown to be elevated in the serum, and local production of IgE in tears has been postulated.[10–14] However, levels of tear IgE were low, at 18.5 ± 19.2 U/ml, in our patients and we never observed a positive RAST in tears in the presence of negative results in the serum.[15]

Recent studies suggest a more complex non-IgE-dependent pathogenic mechanism. A multitude of cells and mediators have been detected in the serum, conjunctiva, and tears of patients with VKC, which may have a relevant role in the pathogenesis of the disease.[12,16–19] Although many features of VKC suggest an allergic pathogenesis,[3,12,20] this eye disease can no longer be considered a classical type I IgE-mediated disease, as included in the classification by Gell and Coombs.[16,21] In fact, skin tests and RAST are often negative in VKC and several patients do not have a personal or family history of atopy.[5] On the basis of challenge studies as well as immunohistochemical and mediator studies, we recently suggested a Th2-driven mechanism and a definition, similar to that of asthma, of 'an allergic inflammatory disease of the conjunctiva with mast cells, eosinophils, and lymphocytes'.[21] Supporting this definition are the findings that T-cell clones derived from VKC tissues are mainly of the Th2- type and that in the CD4 areas of VKC biopsies, there is an increased *in situ* hybridization signal for IL-5 associated with increased IL-5, but not IL-2, levels in tears, suggesting Th2 rather than Th1 activity.[18,22–25] It is possible that the pathogenesis of VKC is characterized by a Th2 lymphocyte alteration, while the exaggerated IgE response to common allergens is an inconsistent and, perhaps, secondary event. Th2 lymphocytes are responsible both for hyperproduction of IgE (interleukin 4, IL-4) and for differentiation and activation of mast cells (IL-3) and eosinophils (IL-5). Mast cells and basophils cause the immediate reaction (through the release of histamine) and the recruitment of inflammatory cells (lymphocytes and eosinophils). This cell recruitment (favoured by an overexpression of adhesion molecules) results in the release of other toxic cell mediators (such as eosinophil cationic protein, EDN/EPX) with corneal epithelial damage.[18,26–28] Indeed, several inflammatory and epithelial cells may induce fibroblast proliferation and collagen production leading to the characteristic conjunctival findings.

While histamine is the main mediator in allergic reactions with a prevalent Type I hypersensitivity mechanism (such as perennial or seasonal allergic conjunctivitis), and in the early phase of allergic reactions (antihistamines are thus effective in these conditions), other mediators are involved in VKC, such as eosinophilic mediators and substances derived from the metabolism of arachidonic acid (prostaglandins and leukotrienes).[13,29] In particular, leukotrienes are produced during allergic and inflammatory respiratory diseases by mast cells, macrophages, and neutrophils.[30,31] They are potent mediators of hypersensitivity and inflammatory reactions.[16] Their activities include smooth-muscle contraction, small vessel dilatation, increase in blood vessel permeability, promotion of glycoprotein secretion from epithelial glands, and increase of nasal blood flow and airway resistance.[32–36] It has been demonstrated that leukotrienes are also produced in the conjunctiva[37] and are detectable in tear fluids of patients affected by allergic conjunctivitis including VKC.[38–40] Indeed LT concentration in tears increases in allergic subjects following allergen challenge.[41,42] In addition, conjunctival administration of LTB4, as well as LTC4 and LTD4, induces vessel vasodilatation, oedema, hyperaemia, and leucocyte and eosinophilic infiltration of the conjunctiva.[43–45] The biological activities of leukotrienes on the conjunctiva may contribute to the presence of the characteristic symptoms observed in VKC, such as mucous secretion, conjunctival hyperaemia, and chemosis.

Neural and endocrine involvement

The existence of a relationship between the central nervous system and the eye is easily established when considering common anatomical and embryogenic origins. This cross-talk is also strictly related to the immune system and is shown by nerve and mast cell interaction. Whether neurotransmitters and neurotrophins may influence conjunctival inflammation is not clear at present. Substance P, a neuropeptide with well-known activity on immune cells, has been detected in tears, and high serum levels have been found in patients with VKC.[46,47]

Receptors for nerve growth factor have been found in the epithelium and substantia propria of the conjunctiva, and high serum levels of nerve growth factor are detectable in the active form of the disease and are directly related to the number of mast cells in the conjunctival tissue, suggesting that neural influences may have a role in the pathogenesis of allergic diseases.[48,49]

A role for sex hormones has been postulated in the pathogenesis of the disease. This assumption derives from the observation of a prevalence of males *vs* females and a spontaneous resolution of the disease at puberty. Sex hormones may play a relevant role in the pathophysiology of allergic diseases by reciprocal interactions between the immune and the endocrine

system. Oestrogens and progesterone have been shown to be active players in the ocular immune system, with an already well-established role in another immunological disease, dry eye syndrome.[50] In a previous immunohistochemical study of patients with VKC, we reported that oestrogen and progesterone receptors were overexpressed on the conjunctiva by eosinophils and other inflammatory cells.[51] These hormones may bind to conjunctival receptors and exert a proinflammatory effect through the recruitment of eosinophils to the conjunctival tissue.[52,53]

Diagnosis

The typical, characteristic signs and symptoms of this disease render the diagnosis of VKC fairly straightforward, even for the general ophthalmologist. Atypical presentations or incomplete forms of VKC may, however, lead to an underestimation of its incidence. The identification of both the major and minor signs and symptoms of VKC allows an early and accurate diagnosis of this disease.

At present, total and specific IgE determination, as well as skin tests cannot be considered useful additional laboratory tests, because more than 50% of patients with VKC are negative.

In case of a diagnostic dilemma, a conjunctival scraping can be precious in demonstrating the presence of eosinophils infiltrating the conjunctival epithelium.

Therapy

Clinical observation suggests that VKC generally subsides with the onset of puberty, but some therapeutic measures may be required beyond this age to control the course of the disease. In some cases, permanent changes to the ocular surface may occur and be accompanied by permanent visual impairment. Although vernal keratoconjunctivitis generally has a good prognosis, 52% of patients in our cohort had persistent symptoms after a mean follow-up period of approximately 5 years and 6% of patients showed a permanent reduction in visual acuity as a result of corneal damage.[5]

Treatment is symptomatic and topical eye drops are generally preferred as first choice. Cromolyn and the new generation of antiallergic compounds such as alomide tromethamine, nedocromil sodium, spaglumic acid, and topical antihistamines are effective in reducing signs and symptoms of the disease.[54] Use of unpreserved solutions may reduce the risk of hypersensitivity to preservatives that are frequently superimposed in these patients. Nonsteroidal anti-inflammatory agents also produce a beneficial effect on the course of VKC.[55] Topical steroid preparations are, naturally, the most effective therapy for moderate to severe form of VKC,[5,9] however their use should be strictly limited and carefully monitored because long-term use of topical steroids is responsible for the 2% incidence of glaucoma in VKC patients.[5]

Therefore, this class of drugs should be used to restore corneal damage induced by epithelial toxic mediators from eosinophils and neutrophils. Once the acute phase of the disease is controlled, steroids should be discontinued and alternative topical treatment (mast cell stabilizers, antihistamine, NSAIDs) should be started.

Cyclosporine A (CsA) from 0.5 to 2% ophthalmic emulsions in olive or castor oil, used four times daily, represents a valid alternative to steroids in severe forms of VKC.

In fact, CsA is effective in controlling ocular inflammation, blocking Th2 lymphocyte proliferation, and IL-2 production. It also inhibits histamine release from mast cells and basophils and, through a reduction of IL-5 production, it may reduce the recruitment and the effects of eosinophils on the conjunctiva.[56–59] Moreover, the therapeutic efficacy of CsA in VKC, a conjunctival hyperproliferative disorder, seems to be related to the drug's efficacy in reducing conjunctival fibroblast proliferation rate and IL-1β production.[60]

In a few patients with VKC, a systemic treatment may be required. Oral antihistamines can reduce the generalized hyper-reactivity but have little or no effect on vernal keratoconjunctivitis,[61] while aspirin treatment,[62,63] as well as oral administration of Montelukast (5–10 mg once/day),[64] an anti-leukotriene drug usually used in mild asthma, has been demonstrated to be effective in reducing signs and symptoms of VKC.

Outcome

Patients with VKC generally have spontaneous resolution of the disease after puberty without any further symptoms or visual complication. However, corneal ulcers, which are reported to develop in approximately 9.7% of patients, as well as the development of cataract or glaucoma, can produce a permanent visual impairment.[65,66]

As negative prognostic factor, it has been reported that the size of the giant papillae is directly related to the probability of the persistence or worsening of symptoms and that the bulbar forms of VKC have a worse long-term prognosis than the tarsal forms.[5]

Conjunctival fibrosis observed on the upper tarsal conjunctiva can be considered to be the natural evolution of giant papillae. It is important to note that in the few patients treated with cryogenic surgery to reduce

papillary excrescencies, a marked pemphigoid-like appearance was evident all over the conjunctiva. Thus, it appears that in some VKC patients, any additional inflammation such as that induced by cryogenic therapy may result in an exaggerated tissue response.

Conclusion

Recently, new reports have improved the knowledge of the pathogenetic mechanisms of VKC. The typical male pattern of incidence significantly decreases with age, with disparity diminishing greatly in patients after puberty. It was shown to be a perennial and not a seasonal disease in the majority of patients, with a tendency to become chronic in long-lasting forms. VKC was shown to be not solely IgE-mediated, but its pathogenesis is multifactorial, mediated by Th2 lymphocytes, eosinophils, IgE, mast cells, and a complex network of interleukins and cell mediators. The presence of increased serum levels of cytokines, enzymes, eosinophil-derived mediators, neuropeptides, and neurotrophins also suggests that this is a systemic, and not just a local, disease.[67–69] The long-term prognosis of the VKC patient is generally good; however approximately 6% of the patients develop a visual impairment owing to corneal damage, cataract, or glaucoma.[5]

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Enhanced Delivery of Ganciclovir to the Brain through the Use of Redox Targeting

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Enhanced delivery of ganciclovir to the brain was demonstrated by a redox-based chemical delivery system. A ganciclovir monoester in which a 1-methyl-1,4-dihydronicotinate was covalently attached to one of the hydroxymethyl functions was prepared. The stability of the ganciclovir chemical delivery system (DHPG-CDS) was evaluated in aqueous buffers and organ homogenates. In vivo distribution studies in the rat indicated that while ganciclovir poorly penetrated into the central nervous system and was rapidly eliminated, DHPG-CDS provided for therapeutically relevant (2.7 μM) and sustained levels of the parent compound through 6 h. An analysis of the area under the concentration curve indicated that the chemical delivery system delivered five times more ganciclovir than that of the parent drug. The high levels in the brain and reduced levels in the blood gave a brain-to-blood drug concentration ratio of 2.54 for ganciclovir when delivered by the chemical delivery system, compared to a ratio of 0.063 when the parent drug was administered. These data suggest that DHPG-CDS could be a useful adjunct for the treatment of cytomegalovirus encephalitis.

Cytomegalovirus (CMV) is a herpesvirus which, in common with other members of this group, leads to a latent disease state during which the viral genome becomes incorporated into the host DNA (19, 21). While CMV infections are common, they are usually benign. There are, however, several circumstances in which CMV infection can product high morbidity and mortality, including in infants and in immunocompromised individuals. The largest upsurge in human CMV infection has been related to the modern plague of AIDS, in which immunosuppression is a hallmark. Human CMV occurs in 94% of all patients suffering with AIDS and has been implicated as a deadly cofactor (11, 33). The virus is thought to be responsible for several clinical presentations, including retinitis (31), pneumonitis (28), and encephalitis (29, 35). Neural involvement of human CMV is widespread but can be proven only by biopsy or postmortem examination. The defining histological evidence for CMV infection in the brain is microglial nodules (18). While central transmission is common, the neurotropic aspects of the causative AIDS pathogen, human immunodeficiency virus type 1, often complicate a clear interpretation of the central cytomegalic disease. In general CMV is thought to produce a subacute encephalopathy similar to that seen with human immunodeficiency virus.

A significant advance in the treatment of human CMV infection came with the discovery and development of ganciclovir, a hydroxymethyl analog of acyclovir (2, 26, 30). Ganciclovir inhibits human CMV reproduction through the action of its triphosphate anabolite which prevents viral DNA replication at the level of DNA polymerase. Administration of ganciclovir to infected patients has demonstrated dramatic improvements in retinitis, with significant reduction in viral titers and clinically important improvements in sight (16, 31). Studies on the treatment of encephalitis have not been encouraging (13, 22). The poor performance of ganciclovir against encephalitis is no doubt related to the low lipophilicity of the drug and its inability to efficiently penetrate various biological membranes, including the blood-brain barrier.

The foregoing discussion suggests that improved therapy for human encephalitic CMV infection might be obtained by increasing the concentration of the drug at its site of action, i.e., the central nervous system (CNS). Such improvement might be achieved through the use of a brain-targeting chemical delivery system (CDS) (5, 6, 8). This technology involves the covalent attachment of a redox targetor to the compound of interest, which provides for an increase in brain uptake due to enhanced lipophilicity. Unlike simple prodrugs, however, the targetor is designed to undergo an enzymatically mediated oxidation that converts the membrane-permeating transport system into a hydrophilic, membrane-nonpermeating conjugate. This polar conjugate is readily eliminated from the systemic circulation but is somewhat retained behind the blood-brain barrier, generating a favorable brain-versus-blood drug concentration ratio as a function of time. The "lock-in" conjugate can then hydrolyze, releasing the parent drug with some selectivity in the CNS. While a variety of targetors have been examined, derivatives of the dihydronicotinate-nicotinate redox couple have proven to be the most successful. Application of the approach to a number of drugs, including antiviral nucleosides, has been reported (1, 4, 7, 9, 10, 24, 25).

MATERIALS AND METHODS

Drugs and chemicals. Microcombustion analyses of compounds prepared were performed by Atlantic Microlabs (Atlanta, Ga.). Uncorrected melting points were determined with an Electrothermal melting-point apparatus. UV spectra were recorded on either a Hewlett-Packard 8451A diode array or a Shimadzu UV-160 rapid-scan spectrophotometer. Nuclear magnetic resonance spectra (NMR) were obtained with a Varian VXR-300 (300-MHz, FT mode) spectrometer. Samples were dissolved in an appropriate deuterated solvent, and chemical shifts (δ) were reported relative to an internal standard (tetramethylsilane). Solvents and reagents were ob-

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tained from Baxter Scientific or Aldrich Chemical Co. Ganciclovir was a gift from Syntex, Inc.

(i) **9-[(3-Hydroxy-1-*tert*-butyldimethylsilyloxy-2-propoxy)methyl]guanine (compound 2 in Fig. 1).** To a solution of 4.5 g (17.63 mmol) of ganciclovir (compound 1 in Fig. 1) in 500 ml of dimethylformamide, 4 g (26.54 mmol) of *tert*-butyldimethylsilyl chloride and 3 g (44.06 mmol) of imidazole were added. The mixture was stirred at 50°C for 16 h and at 25°C for 7 days. Additional amounts of imidazole (3 × 0.75 g) and *tert*-butyldimethylsilyl chloride (3 × 1.0 g) were added during this time to complete the reaction as indicated by the disappearance of ganciclovir. The solvent was reduced in vacuo to approximately 50 ml, and the residue was diluted with water. The formed precipitate was filtered, washed with water, and dried. The crude material, which consisted of a mixture of the monosilyl (compound 2) and bissilyl (compound 2a) derivatives was subjected to open-column chromatography (150 g of silica gel [Davisil] grade 634, 100/200 mesh, 60-Å [6.0-nm] pore size). The monosilyl derivative was obtained with a mobile phase containing chloroform and methanol (85:15). This gave 3.0 g of product (yield = 48%): 245 to 247°C mp; ^{1}H NMR (dimethyl sulfoxide [DMSO]-d_6) δ 7.80 (s, 1H, C-8 proton), 6.50 (bs, 2H, NH_2), 5.40 (bs, 2H, C-1' proton), 4.70 (m, 1H, C-3' proton), 3.50 (m, 3H, C-4' and C-5' protons), 0.90 [s, 9H, $C(CH_3)_3$], 0.10 [s, 6H, $Si(CH_3)_2$].

(ii) **3-[[9-(3-Hydroxypropoxy)methyl]guanidinyl]-1-methylpyridinium iodide (DHPG-Q$^+$, compound 4 in Fig. 1).** To a solution of 1.87 g (5 mmol) of compound 2 in 100 ml of dry dimethylformamide, a mixture of 2.21 g (5 mmol) of the mixed anhydride 3-(2,6-dichlorobenzoyl)carbonyl-1 methylpyridinium iodide (compound 3 in Fig. 1) and 0.61 g (5 mmol) of 4-dimethylaminopyridine was added. The mixed anhydride (compound 3) was prepared by reacting nicotinic acid with 2,6-dichlorobenzoic acid in benzene with triethylamine as a proton scavenger, and then the resulting anhydride was quaternized with methyl iodide in acetonitrile. The mixture of compounds 2 and 3 was stirred at 20 to 25°C for 2 days with exclusion of moisture and under argon. The precipitated solid (2,6-dichlorobenzoic acid) was removed by filtration. To the filtrate was then added 3.30 g (12.6 mmol) of tetrabutylammonium fluoride, and the system was stirred for 2 days at 20 to 25°C. The solvent was removed in vacuo to leave a gum that was triturated with acetone. The solid produced was filtered and used without further purification.

(iii) **9-[1-[[(1,4-Dihydro-1-methylpyrin-3-yl)carbonyl]oxy]-3-hydroxy-2-propoxymethyl]guanine (DHPG-CDS, compound 5 in Fig. 1).** DHPG-Q$^+$ (2.55 g, 5 mmol) was dissolved in 100 ml of cold, degassed water. To this solution was added 2.56 g (30 mmol) of sodium bicarbonate and 3.53 g (20 mmol) of sodium dithionite. The mixture was stirred for 1 h, at which time the formed precipitate was removed by filtration, washed with cold water, and dried in vacuo, giving 0.8 g of DHPG-CDS: 198°C mp (dec.); high-performance liquid chromatography (HPLC), 98%; ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1H, C-8 proton), 6.8 (d, 1H, dihydropyridine C-2 proton), 6.50 (bs, 2H, NH_2), 5.80 (m, 1H, dihydropyridine C-6), 5.50 (bs, 2H, C-1' proton), 4.80 (m, 1H, dihydropyridine C-5 proton), 4.00 (m, 2H, C-3' and C-4' protons), 3.50 (bs, 1H, OH), 3.00 (m, 5H, dihydropyridine C-4 and N-CH_3 protons). Analysis calculated for $C_{16}H_{20}N_6O_5 \cdot 3/2H_2O$: C, 47.64%; H, 5.62%; N, 20.83%. Found: C, 47.92%; H, 5.63%; N, 20.63%.

Analytical methodology. HPLC was used in the detection, separation, and quantitation of the compounds of interest. The chromatographic system consisted of a Perkin-Elmer Series 4 microprocessor-controlled solvent delivery system, a Perkin-Elmer ISS-100 autosampler, a Kratos Spectroflow 757 UV/VIS variable-wavelength detector, and a SpectraPhysics model 4290 integrator. Compounds were chromatographed on an analytical column (25 cm by 4.6 mm [inside diameter]; Spherisorb ODS-2; 5-μm particle size; Alltech, Inc.) fitted with a guard column and a 2-μm-pore-size in-line filter. All determinations were performed at ambient temperature, and analytes were detected at 254 nm. For buffer and homogenate analyses, the mobile phase used to elute DHPG-Q$^+$ and ganciclovir contained 10 mM KH_2PO_4-acetonitrile (4:1) flowing at 1.0 ml/min. Under these conditions, DHPG-Q$^+$ eluted at 5 min while ganciclovir had a retention time of 3 min. For DHPG-CDS, a mobile phase containing water-acetonitrile (80:20) flowing at 1.0 ml/min was used. For DHPG-CDS, the retention time was 5.2 min in this system. For analysis of ganciclovir and DHPG-Q$^+$ in biological tissue derived from the tissue distribution studies, a different method was applied. In this system, the mobile phase contained 95% 10 mM KH_2PO_4, 5% methanol, and 1.0 mM tetramethyl ammonium perchlorate as a competing salt. Ganciclovir and the corresponding quaternary salts had retention times of 8 and 14.5 min, respectively, in this system. Standard curves were linear over the concentration range of interest ($r > 0.999$). The limits of accurate quantitation of ganciclovir in the biological tissues examined (rat brain, lung, and blood) were 50 ng/ml or g (coefficient of variation, 5.2%) and 275 ng/ml or g for DHPG-Q$^+$ (coefficient of variation, 6.3%).

Physicochemical and stability studies. The apparent partition coefficients (PCs) for ganciclovir, DHPG-Q$^+$, and DHPG-CDS were determined in an octanol-water system (23). After equilibration of *n*-octanol in water, the drugs of interest were dissolved in the appropriate phase (DHPG-CDS in octanol and DHPG-Q$^+$ and ganciclovir in the aqueous phase). Typically, 0.5 ml of the drug solution was allowed to equilibrate with the second phase by using various volumes (5 to 15 ml). The phases were then separated by centrifugation (7,000 rpm for 2 min), and the concentration of the object compounds was determined by HPLC as described above. The stability of DHPG-CDS and the corresponding quaternary salt was determined in a number of buffer systems over a wide pH range (pH 4 to 10). All studies were completed at 37°C at constant ionic strength (μ = 0.01 M). The buffers used were acetate in the pH range of 4 to 5, phosphate in the pH range of 6 to 8, and carbonate in the pH range of 9 to 10. At pH values of <7, the rate of DHPG-CDS was monitored by UV spectrophotometry of the 360 band III absorbance. Kinetic determinations were made with the aid of a dedicated HP 85 microprocessor and a data acquisition and manipulation software program written for the computer. Kinetic experiments were performed using a 2.5-ml portion of the buffer maintained in a thermostated cell holder. A concentrated solution of the compound of interest was prepared in DMSO, and a portion (25 μl) was added to the buffer to give an initial drug concentration of 50 μM. At pH values of >7 for DHPG-CDS and for all studies involving DHPG-Q$^+$, the rate of degradation in buffer was monitored by HPLC using the method described above. In this series of experiments, a 50-μl aliquot of a DMSO solution of the compound of interest was added to 5 ml of the appropriate buffer maintained in a thermostated water bath. As in the UV experiments, the initial drug concentration was 50 μM. At various times after drug addition to the buffers, samples were withdrawn and analyzed. In both the UV and HPLC experiments, pseudo-first-order conditions were maintained. Rate constants were obtained from the slope of a plot of either log peak height or log absorbance as a function of time. In these experiments, the disappearances of DHPG-CDS and DHPG-Q$^+$ were monitored as were the appearances of

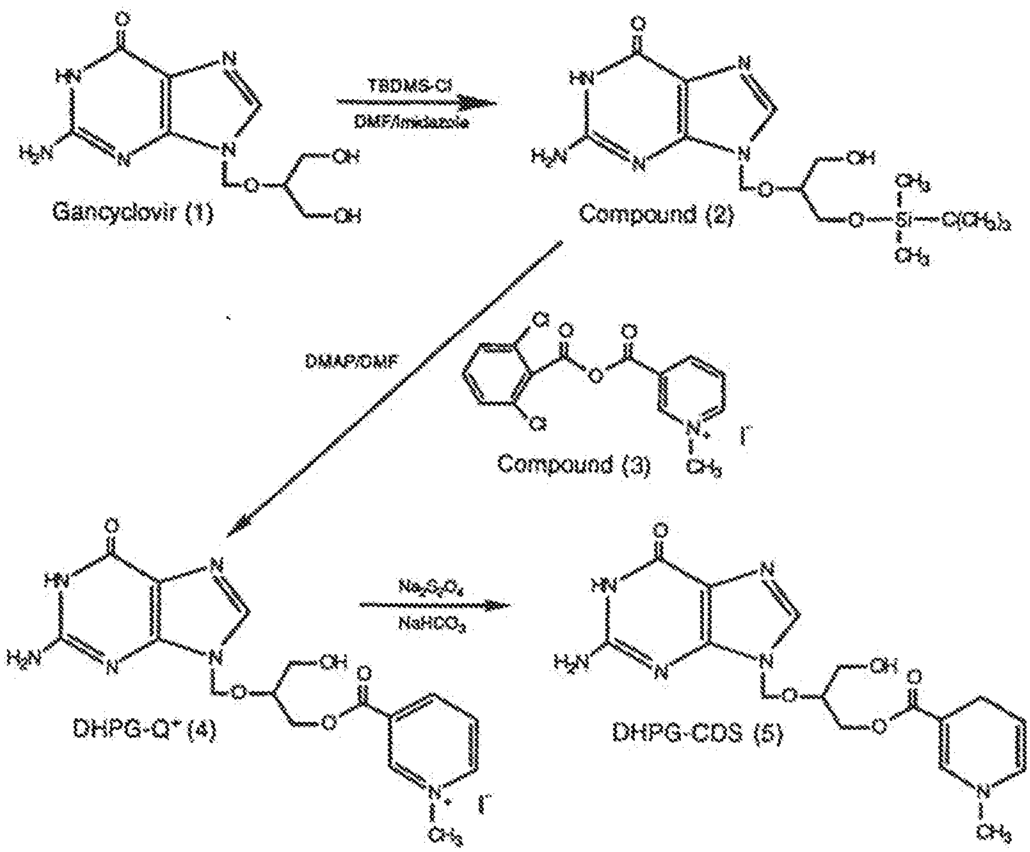

FIG. 1. Synthesis of DHPG-CDS as well as the precursor quaternary salt (DHPG-Q$^+$).

DHPG-Q$^+$ and ganciclovir. Degradation of ganciclovir to guanine was not modeled in our analysis. All experiments monitored buffer-induced degradation over at least three half-lives ($t_{1/2}$s). For in vitro homogenate studies, freshly obtained rat (Sprague-Dawley) tissues (blood and brain) or human blood was used. Brain tissue was homogenized with a Potter-Elvenjen glass tube and Teflon pestle in cold isotonic phosphate-buffered saline to give a 20% (wt/vol) homogenate. Blood or brain homogenate was then placed in a Teflon-lined screw-top vial and equilibrated at 37°C. A DMSO solution of the appropriate compound was then added to the biological matrix to produce an initial concentration of 50 μM. At various times after the addition, 200 μl of the sample was removed and added to 800 μl of ice-cold acetonitrile. The suspension was centrifuged at 10,000 rpm for 3 min. The supernatant was withdrawn, filtered through a 0.45-μm-pore-size syringe filter (Millipore, Inc.), and analyzed by HPLC as described above. Kinetics of degradation for the systems described were pseudo-first order, and rate constants (k) were determined from the negative of the slope of a natural log plot of the change in chromatographic peak height with time. $t_{1/2}$s were calculated as (ln 2)/k.

Tissue distribution in rats. Conscious Sprague-Dawley rats (body weight = 200 g) were restrained in a Broome-type holder and were injected intravenously via the tail vein with either ganciclovir or DHPG-CDS. The vehicle for both compounds was DMSO (0.5 ml/kg of body weight), and the drug dose was maintained at 80 μmol/kg (20 mg of ganciclovir per kg and 30 mg of DHPG-CDS per kg). At various times subsequent to drug administration (15 and 30 min and 1, 2, 4, 6, and 24 h), animals were sacrificed by rapid decapitation and brains, lungs, and trunk blood were collected. The blood was collected into heparinized tubes and centrifuged at 3,000 rpm for 10 min, and the plasma was separated and stored at −20°C. Organs were collected, weighed, and stored on dry ice prior to sample preparation. Plasma samples (1.0 ml) were then diluted with 4 ml of a mixture of equal volumes of acetonitrile and 0.01 M phosphate buffer (pH 3.2) and centrifuged at 12,000 rpm for 15 min, and the supernatant was separated and filtered for HPLC analysis. The organs (1 to 2 g) were homogenized in a mixture of acetonitrile and phosphate-buffered saline and centrifuged at 12,000 rpm for 15 min. The supernatant was filtered and analyzed by HPLC. Area under the curve analyses were done using the RSTRIP software package (MicroMath, Inc.)

RESULTS

Chemistry. DHPG-CDS was synthesized according to Fig. 1 in which ganciclovir was reacted with *tert*-butyldimethylsilyl chloride to give equal quantities of the monosilyl and bisilyl protected compounds. This mixture was separated by preparative open-column chromatography to give the pure monosilyl protected ganciclovir derivative. The 1-methylnicotinate ester was then attached in a single step using the novel acylating agent 1-(2,6-dichlorophenyl)-3-(1-methyl-3-pyridinyl)carbonic anhydride. The development of this reagent was necessary since methylation of a nicotinate ester would likely be complicated by side reactions, specifically guanine N^7 alkylation. During reaction workup the protecting silyl group was re-

TABLE 1. k_{obs}, $t_{1/2}$s, and correlation coefficients (r) for decomposition of DHPG-CDS and DHPG-Q^+ in aqueous buffers

Drug	pH	k_{obs} (min^{-1})	$t_{1/2}$ (min)	r
DHPG-CDS	4.00	0.0445	15.29	0.990
	4.51	0.0208	33.16	0.993
	5.00	0.0078	87.89	0.997
	6.00	0.0042	164.02	0.995
	6.63	0.0029	236.23	0.996
	7.04	0.0018	384.60	0.998
	7.40	0.0012	540.77	0.999
	8.15	0.0008	840.62	0.998
DHPG-Q^+	6.00	0.0002	2,783.70	0.985
	7.04	0.0018	374.99	0.997
	7.40	0.0053	128.59	0.989
	8.15	0.0204	33.90	0.988
	9.08	0.1371	5.05	0.999
	10.00	0.6671	1.04	0.996

moved, yielding DHPG-Q^+, which upon sodium dithionite reduction gave rise to DHPG-CDS.

Lipophilicity. The octanol-water PCs for ganciclovir, DHPG-Q^+, and for DHPG-CDS were determined by traditional shake-flask methods. The log PC for ganciclovir was found to be −1.95, consistent with previously determined values (3, 20). DHPG-CDS was found to be 55 times more lipophilic than ganciclovir (log PC = −0.20), while DHPG-Q^+ was more than twofold less lipophilic (log PC = −2.33).

Stability in buffers. The stability of both DHPG-Q^+ and DHPG-CDS was determined in a variety of buffer systems, with results reported in Table 1 and Fig. 2. The logarithms of the observed rate constants (k_{obs}) were linear as a function of pH for the quaternary salt and the dihydronicotinate. The following equations were generated: $\log k_{obs} = 0.116 - 0.405$ pH (DHPG-CDS) and $\log k_{obs} = -8.93 + 0.883$ pH (DHPG-Q^+). Since extrapolation to zero buffer concentration was not performed, the rate values include in their magnitude both specific and potentially general catalytic terms. Given this caveat, it is clear that the stability of the CDS decreases as the pH decreases while DHPG-Q^+ increases in stability with an increase in pH. This profile is consistent with the documented acid lability of dihydronicotinate, which typically undergoes acid-catalyzed irreversible water addition across the 5,6-double bond, resulting in the formation of a 6-hydroxytetrahydropyridine species. The acid-catalyzed degradation product is detected in the buffer samples. In the case of DHPG-Q^+, base-catalyzed ester hydrolysis is the likely cause for the instability at high pH values.

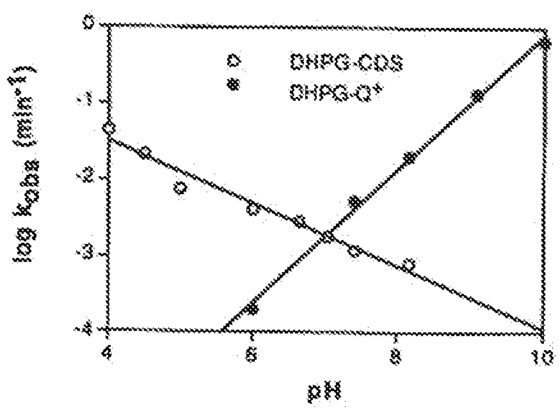

FIG. 2. pH Rate (k_{obs}) profile for DHPG-CDS and DHPG-Q^+ in aqueous buffer solutions.

TABLE 2. k_{obs}s, $t_{1/2}$s, and correlation coefficients (r) for decomposition of DHPG-CDS and DHPG-Q^+ in various biological matrices

Drug	Matrix	k_{obs} (min^{-1})	$t_{1/2}$ (min)	r
DHPG-CDS	Rat blood	0.0127	54.54	0.905
	Rat brain (20%)	0.0803	8.63	0.995
	Human blood	0.0231	29.95	0.928
DHPG-Q^+	Rat blood	0.0154	44.96	0.993
	Rat brain (20%)	0.0204	33.95	0.991
	Human blood	0.0132	52.30	0.982

Stability in tissue homogenates and blood. Results of the stability studies in rat brain homogenates and human and rat blood are given in Table 2. The data indicate rapid oxidation of DHPG-CDS in 20% rat brain homogenate, with a measured $t_{1/2}$ of 8.6 min. This represents a substantial acceleration of the degradation rate observed in pH 7.4 phosphate buffer ($t_{1/2}$ = 9 h) and is indicative of an enzyme-mediated oxidation. Oxidation of DHPG-CDS in whole blood was slower than that observed in brain homogenate, with pseudo-first-order $t_{1/2}$s of 54 and 30 min for rat and human blood, respectively. In these studies, no acid-catalyzed addition product was observed. DHPG-Q^+ was hydrolyzed with a $t_{1/2}$ of between 34 and 52 min in the three biological matrices examined, and the acceleration over the rate of hydrolysis in buffer ($t_{1/2}$ = 2 h) was not as dramatic as that seen for buffer versus enzymatic oxidation of DHPG-CDS.

Tissue distribution studies in the rat. Results of the tissue distribution study of both intravenously administered ganciclovir and DHPG-CDS are presented in Tables 3 and 4 and in Fig. 3. Administration of ganciclovir to rats produced a distributional profile consistent with the polar nature of the drug. High initial levels of drug were observed in the blood and lungs, with poor penetration into the brain. Levels in the blood fell rapidly from an initial value of 22 μg/ml at 15 min to undetectable levels at 2 h. The estimated $t_{1/2}$ of ganciclovir in blood was 30 min (r = 0.94). A similar profile was observed in the lung, with initial high drug levels disappearing by 2 h. In the brain, the concentration of ganciclovir was highest at 15 min but was only 2.5% of the initial levels in blood. The concentrations in the brain rapidly fell below the therapeutic window and were undetectable at 4 h, with an apparent $t_{1/2}$ of disappearance of 60 min (r = 0.88). After administration of DHPG-CDS a significantly different profile was obtained. DHPG-Q^+ was observed in high concentrations in both the lung and blood, indicative of extensive tissue distribution by DHPG-CDS (Table 5). Detectable levels of the quaternary salt which were

TABLE 3. Levels of ganciclovir in various tissues after administration of a 20-mg/kg (80-μmol/kg) dose of ganciclovir

Time (h)	Ganciclovir level[a] ± SEM in:		
	Plasma (μg/ml)	Lung (μg/g)	Brain (μg/g)
0.25	22.01 ± 2.08	21.59 ± 2.35	0.60 ± 0.05
0.50	6.59 ± 1.53	10.35 ± 0.53	0.33 ± 0.04
1	3.32 ± 0.60	6.88 ± 0.92	0.20 ± 0.02
2	----	----	0.16 ± 0.05

[a] ----, greater than the limit of detection but less than the limit of quantitation. At 4, 6, and 24 h levels in all tissues were lower than the limit of detection.

TABLE 4. Levels of gancyclovir in various tissues after administration of a 30-mg/kg (80-μmol/kg) dose of DHPG-CDS

Time (h)	Ganciclovir level[a] ± SEM in:		
	Plasma (μg/ml)	Lung (μg/g)	Brain (μg/g)
0.25	1.35 ± 0.05	0.53 ± 0.03	0.69 ± 0.03
0.50	0.82 ± 0.04	0.51 ± 0.06	0.71 ± 0.01
1	0.37 ± 0.04	0.43 ± 0.05	0.63 ± 0.03
2	0.33 ± 0.02	0.32 ± 0.04	0.49 ± 0.02
4	0	0.27 ± 0.04	0.65 ± 0.04
6	0	0.23 ± 0.02	0.66 ± 0.07

[a] 0, lower than the limit of detection. At 24 h, levels in all tissues were lower than the limit of detection.

TABLE 5. Levels of DHPG-Q$^+$ in various tissues after administration of a 30-mg/kg (80-μmol/kg) dose of DHPG-CDS

Time (h)	DHPG-Q$^+$ level[a] ± SEM in:	
	Plasma (μg/ml)	Lung (μg/g)
0.25	29.48 ± 2.82	7.21 ± 0.53
0.50	17.57 ± 0.61	8.86 ± 0.74
1	15.76 ± 0.18	5.54 ± 1.53
2	15.15 ± 3.50	3.74 ± 0.38
4	8.76 ± 2.41	3.55 ± 0.69
6	5.31 ± 0.53	1.63 ± 0.81

[a] In the brain, levels from 0.25 to 6 h were greater than the limit of detection but less than the limit of quantitation; in all tissues at 24 h levels were lower than the limit of detection.

below the limit of quantitation were also observed in the brain through 6 h. DHPG-Q$^+$ was associated with release of the parent compound in blood, with observed levels between 1.35 and 0.33 μg/ml. These are significantly below the ganciclovir level generated after ganciclovir treatment. By contrast, significantly higher levels of ganciclovir were generated in the brains of rats treated with DHPG-CDS than were generated in the brains of rats treated with ganciclovir. The levels were sustained, with 15-min and 6-h concentrations being almost identical (0.69 versus 0.66 μg/g), indicating potentially therapeutic concentrations throughout the 6-h time course (14). The area under the brain drug concentration curve from 0 to 6 h was five times greater after DHPG-CDS treatment (3.61 μg · h/ml) than after ganciclovir administration (0.664 μg · h/ml). Levels of ganciclovir in the blood were significantly lower after DHPG-CDS treatment than after ganciclovir administration (area under the curve from 0 to 6 h = 10.46 μg · h/ml after ganciclovir treatment and 1.418 μg · h/ml after DHPG-CDS treatment). The increased levels in the brain and decreased concentrations in blood resulted in an increase of the brain-to-blood drug concentration ratio, as measured by comparisons of the area under the curve, from 0.063 in the case of ganciclovir administration to 2.54 in the case of DHPG-CDS administration. This represents a 40-fold increase in the brain/blood drug concentration ratio. All tissue were cleared of drug by 24 h.

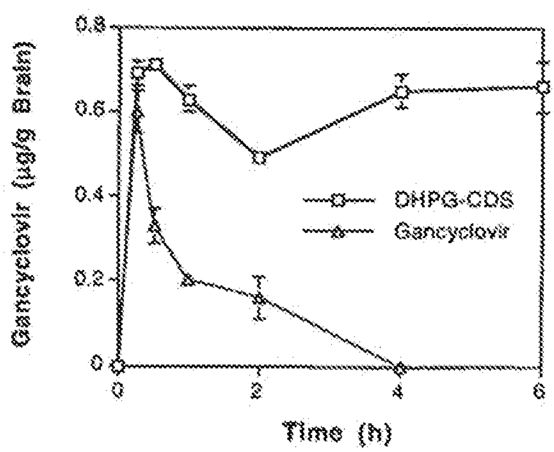

FIG. 3. Brain ganciclovir concentrations as a function of time after an intravenous dose of 80 μmol of either DHPG-CDS or ganciclovir per kg.

DISCUSSION

Human CMV infection is a pernicious opportunistic malady associated with immunosuppression with manifestations in the eye, brain, and lung. While very positive results can be pointed to in alleviation of retinitis, the treatment of encephalitic cytomegalic disease is far less successful with ganciclovir. Thus, Fiala et al. found that while a few patients and specifically those diagnosed with meningoencephalitic disease demonstrated some improvement with ganciclovir, the majority of patients with CNS CMV infection including subacute encephalopathy and polyneuropathies did not improve (13). In addition, some reports indicate that patients became worse when ganciclovir was used to treat brain infections (22).

The inability of ganciclovir to adequately treat central infection is likely related to its inability to penetrate biological membranes. As reviewed by Greig, there are three important factors governing CNS uptake of drugs, including the time-dependent free concentration profile of the compound of interest in plasma; the permeability of the compound through the blood-brain barrier, which is highly correlated with the derivative's lipophilicity or octanol-water PC; and the local blood flow (17). In the case of compound lipophilicity, studies have indicated that compounds with PCs of 1.0 (log PC > 0) are readily transferred across the blood-brain barrier (in the absence of enzymatic instability or other complicating factors) and their uptake is blood flow dependent. PCs of less than 0.1 (log PC < −1.0) are associated with permeability-limited uptake of compounds from brain to blood. Between these values, both blood flow and drug permeability may be limiting. The log PC value of ganciclovir is −2.0, which, as expected, confers poor (nonfacilitative) membrane penetration characteristics. As a result, the oral availability of ganciclovir is low (2.5 to 6%) and its ability to penetrate the blood-brain barrier is very limited. Autopsy studies indicate that ganciclovir concentrations in the brain were only 38% of the levels in cardiac blood and many times lower than the level of drug found in the kidneys (34). Other studies indicated poor uptake of ganciclovir into cerebrospiral fluid (31%) 3.5 h after intravenous infusion (15).

Various attempts have been made to improve the physicochemical properties of ganciclovir, with the most often stated aim of improving oral bioavailability or skin penetration. Thus, Martin et al. prepared a series of diester, ester-ether, and diether prodrugs of ganciclovir and tested the oral effectiveness of these compounds in a murine herpes encephalitis model (27). None of the compounds were dramatically more effective than ganciclovir, with 50% effective doses ranging between 55 and 125% of that for ganciclovir. The dihemisuccinate prodrug of ganciclovir was prepared to improve the

aqueous solubility of the parent compound and was in fact 13 times more water soluble (3). Unfortunately, the esters cleaved very slowly in plasma, precluding the use of this compound as a prodrug. The dipropyl derivative of ganciclovir was identified as a possible candidate for development, with a modestly improved (42%) oral bioavailability over that of ganciclovir, but further work demonstrated that its stability in aqueous solutions was poor (32). The diadamantane ester of ganciclovir was suggested by Powell et al. as a derivative with improved skin-penetrating properties (32). The prodrug exhibited good lipophilicity, solution stability, and esterase conversion to the parent compound. Other recently described ester prodrugs include aminomethylbenzoates, which combine such desirable properties as high water solubility at acid pH values with favorable log PC values and rapid enzymatic hydrolysis (20). Diether derivatives, especially the diisopropyl ether (HOE 602), appear to be useful prodrugs for enhancing oral bioavailability of ganciclovir (37, 38). HOE 602 was shown to increase the number of mice surviving a murine CMV challenge when the drug was dosed orally, and the prodrug was found to increase the area under the plasma ganciclovir concentration curve, compared with that of oral ganciclovir, by almost four times in monkeys (38). None of the derivatives or prodrugs developed, however, were tailored to enhance or sustain delivery to the brain.

Many of the derivatives mentioned above could enhance drug movement into the CNS, if so applied, on the basis of their increased lipophilicity, but this action is not selective. As a result, the extraction of the lipophile by other tissues is also increased relative to that of the unmanipulated drug (36). For toxic or cytotoxic agents this is undesirable since the prodrug often generates a greater tissue burden which often increases the toxicity of the prodrug. In attempting to dissociate these two parameters, we applied the redox-based CDS described by Bodor and colleagues to the delivery of ganciclovir to the brain (5, 6, 8).

The CDS approach requires that a molecular targetor be attached to the drug of interest. Ganciclovir has several potential sites for attachment of the targetor, including the two hydroxymethyl positions as well as the 3-hydroxy (keto-enol) function. While all of the sites mentioned are accessible, the initial design process suggested attachment of a single 1-methyldihydronicotinate targetor group to one of the hydroxymethyl groups. It was expected that this manipulation would provide a sufficient improvement in lipophilicity to allow for blood-brain barrier transit of the conjugate. Other manipulations were not attempted at the onset since they were thought to complicate the kinetic scheme. The designed CDS was prepared by established procedures and tested in vitro and in vivo to assay its ability to act as a brain-targeting delivery form.

The DHPG-CDS was found to be 55 times more lipophilic than the parent compound but still have a log PC value close to zero. This would suggest CNS uptake greater than that of ganciclovir but still partially dependent on molecular permeability (17). The corresponding quaternary salt was two- to threefold more hydrophilic than ganciclovir, consistent with its polar structure. This lower log PC value should aid in the rapid disposition of the DHPG-Q$^+$ which forms in situ from the administered DHPG-CDS. Buffer studies demonstrated good chemical stability of the compounds at neutral pH values. DHPG-CDS is poorly stable in the acid pH range, suggesting that oral delivery systems will require enteric coating to ferry the drug past the stomach to the intestine. DHPG-Q$^+$, on the other hand, is poorly stable in alkaline solutions because of ester hydrolysis. In rat brain homogenate, DHPG-CDS rapidly oxidized to the corresponding quaternary salt, with a $t_{1/2}$ of minutes, while the stability in rat blood lasted approximately 1 h. This suggested reasonable stability in blood during the distributional phase of the CDS and rapid conversion of the compound in brain. The $t_{1/2}$ of hydrolysis of the oxidized salt in brain homogenate was approximately 30 min and suggested facile conversion of the quaternary depot form to the parent agent.

Animal distribution data were supportive of the CDS concept in the case of DHPG-CDS. While uptake of ganciclovir was limited and its concentrations were readily eliminated from the brain, DHPG-CDS provided for sustained brain delivery of ganciclovir through 6 h at concentrations that have been reported to inhibit viral replication in vitro (50% inhibitory concentration = 0.5 to 3 μM; 6-h concentration in the brain = 2.7 μM) (14). The area under the brain drug concentration curves were fivefold higher after DHPG-CDS administration than after ganciclovir administration. The higher levels of ganciclovir in the brain may result from a distributional advantage offered by DHPG-CDS; i.e., the CDS may provide for a partitioning of the ganciclovir into brain compartments deeper than those that are accessible to the unmanipulated drug. Such a pharmacokinetic change would provide for a longer residence time even in the absence of high levels of the DHPG-Q$^+$ precursor. In addition, levels of the parent drug in blood after DHPG-CDS treatment were much lower than those generated by ganciclovir. The lower systemic levels may have important toxicological ramifications. The most often reported dose-limiting side effect of ganciclovir is neutropenia, which affects about 40% of patients taking the drug, followed by thrombocytopenia, which occurs in approximately 20% of all patients taking ganciclovir (12). The lower peripheral levels of ganciclovir produced by DHPG-CDS may therefore reduce the incidence or severity of these hematological reactions.

In summary, DHPG-CDS provides for some improvement in organ selectivity by producing a significant increase in the brain-to-blood and other organ-to-blood drug level ratios as a function of time. DHPG-CDS was found to sustain drug levels in the brain at therapeutically significant levels and to reduce concentrations in the blood compartment, which is associated with toxicity.

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Intravenous and Oral Pharmacokinetic Evaluation of a 2-Hydroxypropyl-β-cyclodextrin-Based Formulation of Carbamazepine in the Dog: Comparison with Commercially Available Tablets and Suspensions

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Abstract □ Complexation of carbamazepine with 2-hydroxypropyl-β-cyclodextrin was performed to provide improved formulations of this widely used antiepileptic drug. Based on this approach, liquid dosage forms were configured for both parenteral and oral use. Intravenous administration of an aqueous carbamazepine-2-hydroxypropyl-β-cyclodextrin (CBZ·HPβCD) complex at a CBZ dose of 20 mg/kg was well tolerated and generated high initial drug levels that fell monoexponentially as a function of time, yielding a plasma elimination half-life of 38 min. Oral studies were completed with three preparations: a commercially available tablet and suspension, as well as a CBZ·HPβCD oral solution. Oral administration of tablets gave erratic and slow absorption, leading to maximum CBZ concentrations (C_{max}) of <2 μg/mL, which were manifested only at 2.5 h after drug dosing. The absolute bioavailability of CBZ from the tablets was ~25%. Both the suspension and CBZ·HPβCD solution gave a significantly improved profile. Thus, the liquid oral dosage forms approximately doubled the oral bioavailability of CBZ compared with the tablets.

Introduction

Carbamazepine (CBZ) is the most widely used antiepileptic drug in the world.[1,2] The popularity of this agent is related to several beneficial clinical properties, including proven efficacy in controlling a number of seizure types, an acceptable side effect spectrum, and a well-characterized pharmacokinetic profile.[3] Although the drug has been successfully used for >20 years, the poor aqueous solubility of CBZ has precluded the development of a commercially available parenteral formulation. The lack of such a dosage form complicates treatment in various instances. Thus, CBZ cannot be currently used in the treatment of status epilepticus, and patients must be switched to other anticonvulsants to control these episodes.[4] Changing drug regimens is complex, however, because antiepileptic drugs in general, and CBZ in particular, induce hepatic enzymes of the P450 family, which results in alterations of the pharmacokinetic profile of CBZ.[5] Polypharmacy may, therefore, give rise to poor seizure management once the status epilepticus crisis has passed. In addition, the lack of a parenteral CBZ product prevents optimal management for the epileptic surgical patient and individuals who cannot take medication orally. Similarly, no simple method of administering loading doses of the drug is available.[4] Finally, a parenteral CBZ formulation would be a valuable tool for assessing absolute bioavailability. It is an interesting observation that even after 30 years of clinical use, the oral bioavailability of CBZ has not been determined by traditional techniques because of the lack of an intravenous (iv) formulation.

It has become increasing apparent that improvements to the solid dosage form of CBZ may also be useful. Various studies have suggested that the bioavailability of CBZ is not complete and that gastrointestinal absorption is erratic.[6–9] The matter is complicated by polymorphism in that the CBZ dihydrate, which forms under conditions of high relative humidity, is less water soluble than the anhydrous substance.[10] Thus, tablets stored in humid conditions often did not provide for adequate seizure control. Alternate oral formulations may, therefore, be of interest.

One method that has increased CBZ aqueous solubility, dissolution rate, and bioavailability is cyclodextrin complexation.[11–13] Cyclodextrin increases water solubility through the formation of dynamic inclusion complexes wherein the hydrophobic drug interacts with the hydrophobic cavity of the cyclic starch.[14] The hydrophilic exterior of the cyclic starch provides for the necessary aqueous solubility. Several cyclodextrins have been considered, and the family of 2-hydroxypropylated derivatives, especially 2-hydroxypropyl-β-cyclodextrin (HPβCD), appears to be useful based on safety in humans and complexation potential.[15,16] Complexes of CBZ with HPβCD increased the water solubility of the drug from 0.01 to >10 mg/mL [at an HPβCD concentration of 22.5% (w/v), which is isotonic].[17] Formulations generated in this way are stable to terminal sterilization and have been stored in solution for well over 2 years without significant degradation. In addition, studies in mice and rats indicate that the anticonvulsant effects of the drug are rapidly manifested after iv administration, suggesting that complexation in no way diminishes drug efficacy.[17,18] Results of bioavailability studies in rats indicate that oral absorption of CBZ from an HPβCD-based formulation was more than twice that obtained from a simple CBZ suspension.[19] In this context, HPβCD acts as a true drug carrier because the starch derivative is not absorbed through the gastrointestinal tract.[16] Data from other studies show that the area under the curve (AUC) for CBZ plasma concentration versus time was significantly higher in the dog after an oral CBZ·HPβCD solution than for commercially available CBZ tablets.[20] Löscher et al.[21] found that iv administration of CBZ·HPβCD at doses of 5 mg/kg were well tolerated and significantly safer than other potential iv systems, such as CBZ solubilized in glycofurol. We present herein the pharmacokinetic behavior of CBZ formulated in HPβCD (CBZ·HPβCD) after iv administration as well as an assessment of three oral treatments (two commercially avail-

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able dosage forms; oral tablets, an oral suspension, and an aqueous CBZ-HPβCD formulation) in a three-way crossover paradigm in the dog.

Experimental Section

Materials—Carbamazepine USP was obtained from Orgamol S.A. (Evionnaz, Switzerland) and HPβCD (degrees of substitution = 7.0) from Roquette Corp. (Gurnee, IL). Carbamazepine 10,11-epoxide (CBZE) was synthesized based on a literature procedure in which CBZ was treated with an excess of *m*-chloroperoxybenzoic acid.[22] The CBZ tablet (200 mg, Tegretol) and suspension (100 mg/5 mL) were obtained from CibaGeneva Pharmaceuticals, (Summit, NJ). Solid complexes of CBZ and HPβCD were prepared as previously described[17] to serve as starting materials for both the liquid oral and parenteral dosage forms. Briefly, 500 g of HPβCD was dissolved in 1.0 L of deionized water, generating a 43.5% (w/v) solution. To this solution was added 50 g of CBZ, and the suspension was stirred for 72 h at room temperature. The system was then filtered, freeze-dried, and subsequently passed through a 60 mesh sieve (particle size ≤ 250 μm). The CBZ content of the complex was determined by high-performance liquid chromatography (HPLC) analysis to be 89 mg of CBZ/g powder. Dilution of the solid complex with water for injection (WFI) afforded a 22.5% (w/v) HPβCD solution (isotonic) containing 10 mg of CBZ/mL. This formulation was used in both oral and iv dosing protocols.

Analytical Methodology—CBZ (both in biological and pharmaceutical matrices) and CBZE were detected and quantitated by HPLC. The system configuration included a Spectra Physics model 8810 solvent pump, a Kratos Spectroflow 757 variable wavelength detector, a Perkin-Elmer model ISS-100 autosampler, and a Spectra Physics model 4270 integrator. Samples were chromatographed on a Supelcosil C-18, 3-μm particle size analytical column (7.5 cm × 4.6 mm i.d.) protected by a Supelguard LC-18-DB (2.0 cm × 4.6 mm i.d.) guard column. The mobile phase contained 25% acetonitrile and 75% phosphate buffer (0.05 M, pH 6). The flow rate was 1.3 mL/min, all determinations were made at ambient temperature, and compounds were detected at 238 nm. Under these conditions, CBZ eluted at 4 min and CBZE had a retention time of 9 min. Concentration–peak area curves (externally standardized) were linear (r > 0.999) over the range of interest (0–50 μg/mL), and limits of quantitation and detection were ~0.05 and 0.015 μg/mL, respectively, for both analytes. The coefficient of variation (CV%) was <5%. The system efficiency was 64 000 theoretical plates/m for CBZ.

For preparation of samples for analysis, thawed dog plasma (400 μL) was treated with 800 μL of ice-cold acetonitrile and vortexed for 30 s. The samples were then centrifuged at 10 000g for 10 min (Beckman Microfuge 12), after which 200 μL of the supernatant were collected in a 2.0-mL autosampler vial. Then, 0.8 mL of phosphate buffer (0.05 M, pH 6) was added to the supernatant. Recoveries of CBZ and CBZE from spiked biological matrices were quantitative (98 ± 4% for CBZ, n = 6; 101 ± 3% for CBZE, n = 6). Urine was allowed to warm to room temperature, and 100 μL was transferred to a 2-mL autosampler vial. To this aliquot was added 0.9 mL of HPLC mobile phase (acetonitrile:phosphate buffer), and then the samples were submitted for analysis. System suitability and limits of detection and quantitation were similar to those reported for plasma.

Animal Protocol—The study was designed to include an iv phase followed by a three-way crossover oral paradigm in which either CBZ tablets, suspension, or an aqueous CBZ-HPβCD solution were administered. Healthy mongrel dogs (n = 5) weighing 15 to 25 kg were acclimatized and conditioned in the core facility at the University of Florida's Department of Animal Resources. Dogs were fasted overnight and provided water ad libitum the night before the study. Dogs were catheterized in each of the two contralateral cephalic veins - one for drug administration and one for blood sampling. Dogs were hydrated with iv lactated Ringer's solution that was administered continuously throughout the experimental procedure. Blood pressures and heart rates were measured noninvasively throughout the experimental session. For the iv portion of the study, each dog received CBZ at a dose of 20 mg/kg in a 22.5% (w/v) HPβCD vehicle containing 10 mg of CBZ/mL. The total HPβCD dose was 500 mg/kg, and the drug solution was administered at 2 mL/Kg as a fast infusion (3.4 mL/min or ~10–15 min/animal). The liquid oral preparations were administered via an orogastic tube, and tablets were administered manually. Intubated animals received two aliquots of water (2 × 125 mL) to ensure complete rinsing of the tube, and animals receiving tablets were administered the same amount of water administered orally with a syringe. In all cases, the dose of CBZ was 20 mg/kg and in all studies, a 2-week washout period was provided to allow for enzyme deinduction.[23] The course of dosing was adjusted so that no two animals received the same order of treatments. Blood was taken prior to drug administration, at the end of the infusion period (for iv treatments), and at 10, 20, 30, 60, 90, 120, 150, 180, and 240 min, and 4, 5, 6, 8, and 24 h subsequent to the end of the infusion period. Heparinized blood was centrifuged immediately, and blood plasma was stored at 0 °C. In addition, urine was collected at various intervals postdosing and frozen.

Pharmacokinetics and Statistics—Drug concentration–plasma profiles were analyzed with a nonlinear regression model.[24] The total areas under the plasma concentration–time curves (AUC_∞) were obtained from the regression program or calculated with the linear trapezoid rule. Other parameters, including the distribution or appearance half-life ($t_{1/2\alpha}$), terminal elimination half-life ($t_{1/2\beta}$), as well as the mean residence time (MRT) were obtained directly from the regression software. Total clearance (CL_T) values obtained as the ratio of dose (D) to total AUC (D/AUC), volume of distribution at steady state (Vd_{ss}) was found as the product of CL_T and MRT, volume of distribution for the central compartment (Vd_{c}) was determined by dividing the dose by the plasma concentration at time 0 (D/C_{p0}), and volume of distribution at pseudo-steady-state ($Vd_{\beta,ss}$) was defined as the ratio of total clearance to the terminal elimination rate constant (CL_T/β).[25] The renal clearance (CL_R) was determined from the slope of a plot of plasma AUC to time t versus total cumulative renal elimination to time t. The significance of differences in the means of calculated pharmacokinetic data was assessed by analysis of variance (ANOVA) with post-hoc Turkey's comparison, with p ≤ 0.05 considered to be statistically significant.

Results and Discussion

The feasibility of parenteral application of CBZ-HPβCD was tested through an examination of the iv safety of the complex at a CBZ dose of 20 mg/kg in the dog. Thereafter, using data from the iv study as a baseline, the absolute oral bioavailability and other pharmacokinetic parameters of three oral preparations of CBZ were assessed. The oral formulations included a commercially available tablet and suspension as well as an oral CBZ-HPβCD solution. In the iv arm of the study, dogs tolerated the 20-mg/kg dose well and there were no clinically significant observations during or after the infusion. No significant changes in heart rate, blood pressure, or respiratory rate were noted, which is consistent with an earlier study (in dogs) in which CBZ-HPβCD was administered iv at a CBZ dose of 5 mg/kg.[21] Intravenous CBZ generated high initial CBZ levels (C_{max} = 32 μg/mL) that fell in a monoexponential fashion as a function of time (Figure 1). The half-life of CBZ in plasma after iv administration of CBZ-HPβCD was 38 min, which is in excellent agreement with the published value of 36 min recorded after a 5-mg/kg dose (Table 1).[21] It should be noted, however, that the terminal elimination phase of the drug in humans is significantly longer than in dogs, with reported values of 20–25 h.[23] CL_T of the drug was ~300 mL/min, and the Vd_{ss} was ~600 mL/kg.

Renal clearance (CL_R) of the drug is 12 mL/min or ~4% of total clearance, which is consistent with the small percentage of unchanged drug recovered in the urine (Figure 2). These data are consistent with the extensive oxidative metabolism of CBZ, which, in the dog, is mainly associated with formation of the stable 10,11-epoxide (i.e., CBZE). Although CBZE is the main metabolite in several animal models, more extensive transformations occur in species that possess significant epoxide hydrolase activity, such as humans.[26] CBZE is produced in plasma with an apparent half-life of 43 min after iv administration of the CBZ-HPβCD solution and is eliminated in a simple first-order fashion, with a plasma half-life of 110 min (Figure 1 and Table 2). The metabolite reaches

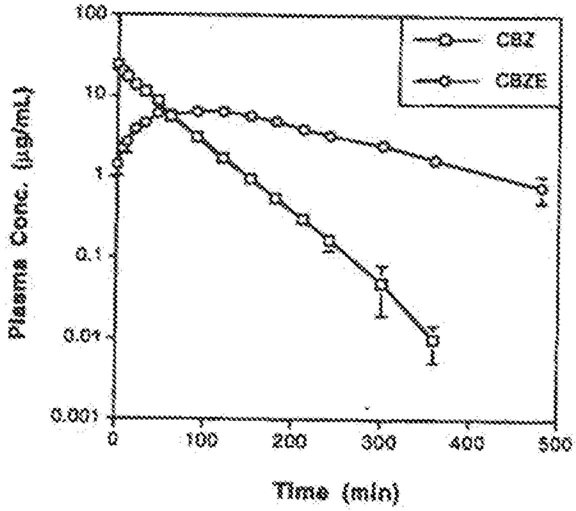

Figure 1—Disposition of CBZ and its 10,11-epoxide metabolite after a 20 mg/kg iv dose of CBZ solubilized in 22.5% 2-hydroxypropyl-β-cyclodextrin. Plasma levels of CBZ were below the limit of detection by 480 min, whereas one of five animals had detectable CBZE levels at 24 h. Data are expressed as means ± SEM (n = 5).

its maximum concentration of 6.46 µg/mL at ~100 min after CBZ administration and is eliminated by the kidneys at a clearance rate of 12 mL/min. Approximately 1% of the administered iv dose was excreted as CBZE (Figure 2). These data are consistent with those determined by Löscher et al. who reported a t_{max} (time to C_{max}) value for CBZE of 81 min after iv dosing with CBZ·HPβCD (5 mg/kg CBZ). In the current investigation, the ratio of the CBZ AUC to the CBZE AUC was 0.76.

Commercially available CBZ tablets demonstrated the poorest pharmacokinetic and bioavailability performance following oral administration. The CBZ AUC after CBZ tablets was 28% of that associated with iv treatment and values were highly variable with a relative standard deviation (RSD) of 71% (Table 1). Peak blood levels were <2 µg/mL and were generated 150 min after dosing (Figure 3). These values for Tegretol tablet dosing in dogs are consistent with previously published studies.[23] Betlach et al.,[30] for example, reported an RSD for AUC values of 78%, a t_{max} value of 63 min, and a C_{max} value of 1.59 µg/mL. The plasma half-life of CBZ after oral tablet administration was 116 min or three-times slower than that observed after iv dosing. The calculated $t_{1/2\alpha}$ of CBZ in plasma was 83 min and CL_R was <1 mL/min (Table 1). The AUC for CBZ was only 28% of that observed after the same dose administered iv, but the plasma AUC for the CBZE metabolite tended to be higher after oral dosing than after iv treatment (Table 2). Peak CBZE levels after tablet administration were ~3 µg/mL and were achieved 310 min after CBZ dosing (Figure 4). The $t_{1/2\alpha}$ of CBZ after CBZ tablet dosing in plasma was 173 min, and the compound was eliminated with a first-order half-life of 246 min. The CL_R of the metabolite was similar to that observed after iv dosing. Taken as a whole, the iv and oral tablet dosing data suggested that the solid dosage form presents less of the drug to the gastrointestinal tract. This finding is consistent with the poor aqueous solubility of CBZ and with the supposition that drug dissolution and/or disintegration is bioavailability limiting.

Oral dosing with either the suspension or HPβCD solution generated a significantly improved kinetic profile compared with that of the CBZ tablets. The AUC values for CBZ after dosing with the suspension or solution were 789 and 732 µg min/mL, respectively, indicating absolute oral bioavailabilities of 51 and 48%, respectively (Table 1). These values are twice as high as those generated by the commercially available tablets. In addition, the data obtained after dosing with the oral suspension or solution demonstrated considerably less variability than did administration of the tablets, with RSDs of 9.3 and 17.8% for oral suspension and solution AUC values, respectively. Maximum CBZ values tended to be higher (4.24 versus 5.35 µg/mL for the suspension and solution, respectively) and t_{max} values shorter (70.4 versus 53 min) for the oral solution compared with the suspension, although the differences in the means did not reach statistical significance (Table 1 and Figure 3). A similar trend was observed with the $t_{1/2\alpha}$ (39 min for the CBZ suspension and 30 min for the CBZ·HPβCD solution). The CL_R was similar not only for the solution and suspension treatments but for all oral dosing paradigms, which is in contrast to the significantly higher values observed for iv dosing. Pharmacokinetic parameters for CBZE after suspension dosing also paralleled those obtained after oral CBZ·HPβCD treatment.

One point raised in earlier work with iv CBZ·HPβCD was that although the HPβCD vehicle was pharmacologically inert, especially when compared with glycofurol or propylene glycol, CBZ levels (as reflected by C_{max} and AUC data) were significantly lower after CBZ·HPβCD dosing as compared with a cosolvent formulation.[21] The reduced plasma levels were apparently associated with faster CBZ elimination from animals treated with the cyclodextrin-containing dosage form. In the current study, elimination of CBZ from plasma subsequent to iv administration was significantly more rapid than elimination after dosing with the CBZ tablet and tended to be faster than that after suspension or solution administration. The rapid loss of iv-administered CBZ from the plasma may be related to several phenomena, including increased CL_R of parenterally administered CBZ and/or slowed elimination of oral administered CBZ due to continued drug absorption during the terminal phase (i.e., flip-flop behavior). In this study, CL_R of CBZ after parenteral dosing was 7 to 14 times higher than that observed after oral dosing. In addition, the cumulative amount of CBZ excreted in the urine (at 8 h after dosing) was 6- to 31-fold higher than that generated after oral dosing. The increase in CL_R associated with iv CBZ·HPβCD is not solely due to the elevated plasma levels of CBZ, which averaged only two to four times higher after iv dosing compared with oral administration. On the other hand, the absolute amount of unchanged CBZ eliminated in the urine was relatively small (~2%), suggesting that another mechanism may well contribute to the relatively high elimination rate of CBZ from blood after parenteral CBZ·HPβCD administration (Figure 2). Increased renal elimination of drugs administered iv in HPβCD has been previously observed and may be related to either facile elimination of the intact complex or recomplexation of the drug at the level of the kidney, resulting in an inhibition of tubular reabsorption.[28] The latter mechanism appears to be more plausible because guest-host decomplexation is a rapid process with complex half-lives on the order of milliseconds. Rapid complex dissociation is also consistent with the observed activity–time profile.[17,18] Recomplexation is possible because at early times after CBZ·HPβCD administration, locally high kidney concentrations of HPβCD and of CBZ are generated and are presumably present in close proximity to each other.[16] On the other hand, the higher elimination rate of parenterally administered CBZ may be a reflection of retarded elimination after oral administration. In the latter case, slowed gastrointestinal adsorption and the resulting flip-flip behavior may be the operant mechanism. The data suggest that both of these actions are ongoing, although the fact that total CBZ elimination increases to only 2% of the administered dose suggests that the flip-flop behavior may be the dominant factor in explaining the differences in drug elimination.

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Table 1—Pharmacokinetic Parameters for CBZ after a 20-mg/kg Dose of CBZ Administered Intravenously and in Various Oral Dosage Forms Including Tablets, A Suspension, and an Oral HPβCD Solution (data are given as mean ± SEM; n = 5)

Parameter	Intravenous	Tablets	Suspension	Solution
AUC_∞ (μg·min/mL)	1536 ± 67^a	425 ± 123^b	789 ± 30^b	731 ± 53^b
RSD (%)	9.65	71.1	9.25	17.8
Bioavailability (%)	—	27.7_a	51.4^b	47.6^b
C_{max} (μg/mL)	32.4 ± 2.1^a	1.64 ± 0.45^b	4.24 ± 0.42^b	5.35 ± 0.51^b
t_{max} (min)	0.00 ± 0.00	148 ± 16^a	70 ± 5^b	53 ± 4^b
Elimination (β) half-life (min)	38 ± 4^a	116 ± 15^b	58 ± 6^a	42 ± 3^a
Appearance (α) half-life (min)	—	83 ± 10^a	39 ± 2^b	30 ± 2^b
MRT (min)	45 ± 5^a	294 ± 33^b	141 ± 12^c	$108 \pm 7^{a,c}$
CL_R	12.1 ± 4.0^a	0.871 ± 0.132^b	1.73 ± 0.23^b	1.74 ± 0.57^b
CL_R (mL/min/kg)	0.531 ± 0.173^a	0.038 ± 0.006^b	0.076 ± 0.010^b	0.076 ± 0.025^b
CL_T (mL/min)	297 ± 18			
CL_T (mL/min/kg)	13.2 ± 0.7			
Vd_{ss} (L/kg)	0.583 ± 0.060			
Vd_{area} (L/kg)	0.725 ± 0.104			
Vd_c (L/kg)	0.554 ± 0.083			

[a–c] Different superscripts indicate significant differences among the means ($p < 0.05$) and similar superscripts indicate no significant difference.

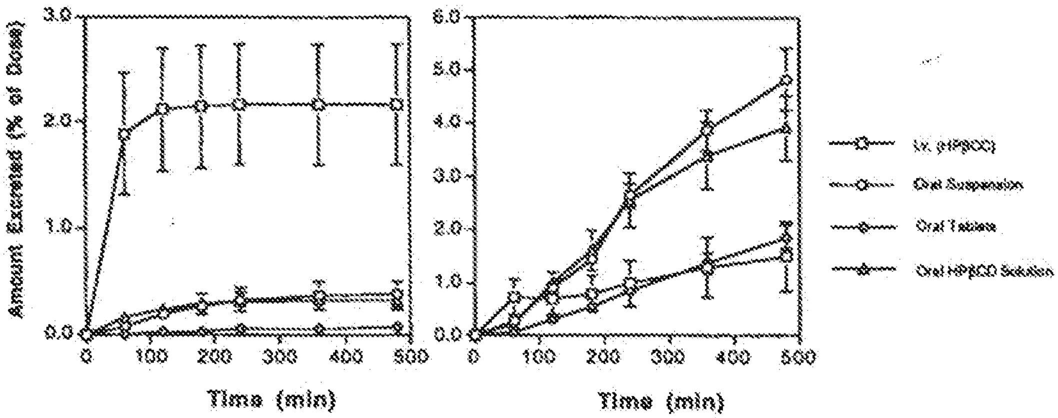

Figure 2—Cumulative amounts of CBZ (left) or CBZE (right) excreted, expressed as percentage of administered dose, after administration of CBZ at a dose of 20 mg/kg given either iv [in 22.5 (w/w) HPβCD] or orally as tablets, a suspension, or an oral solution in HPβCD. Data are expressed as mean ± SEM (n = 5).

Table 2—Pharmacokinetic Parameters for CBZE after a 20-mg/kg Dose of CBZ Administered Intravenously and in Various Oral Dosage Forms Including Tablets, a Suspension, and an Oral HPβCD Solution (data are given as mean ± SEM; n = 5)

Parameter	Intravenous	Tablets	Suspension	Solution
AUC_∞ (μg·min/mL)	2007 ± 279^a	2452 ± 501^a	3035 ± 408^a	2972 ± 313^a
RSD (%)	34.1	50.0	32.9	25.8
AUC_{CBZ}/AUC_{CBZE}	0.76 ± 0.12^a	0.17 ± 0.02^b	0.26 ± 0.03^b	0.25 ± 0.03^b
C_{max} (μg/mL)	6.46 ± 0.58^a	3.14 ± 0.65^b	$4.86 \pm 0.45^{a,b}$	$5.39 \pm 0.37^{a,b}$
t_{max} (min)	101 ± 9^a	310 ± 54^b	$185 \pm 16^{a,b}$	$148 \pm 8^{a,b}$
Elimination (β) half-life (min)	109 ± 11^a	246 ± 35^a	160 ± 17^a	154 ± 31^a
Appearance (α) half-life (min)	43 ± 6^a	173 ± 35^b	$99 \pm 9^{a,b}$	$75 \pm 7^{a,b}$
MRT (min)	225 ± 16^a	621 ± 103^b	$381 \pm 35^{a,b}$	$336 \pm 36^{a,b}$
CL_R (min)	11.7 ± 1.1^a	9.89 ± 1.13^a	14.2 ± 2.4^a	11.4 ± 1.1^a
CL_R (mL/min/kg)	0.514 ± 0.050^a	0.434 ± 0.050^a	0.621 ± 0.106^a	0.502 ± 0.048^a

[a–c] Different superscripts indicate significant differences among the means ($p < 0.05$), and similar superscripts indicate no significant difference.

In conclusion, the data indicate that CBZ can be safely administered iv at doses as high as 20 mg/kg to the dog. Both the drug (at a concentration of 10 mg/mL) and vehicle [22.5% (w/v) HPβCD] were well tolerated under the experimental paradigm employed. These results bolster previously obtained safety data (after a 5-mg/kg iv dose), suggesting that HPβCD could be a useful vehicle for CBZ administration and expand the potential iv dose ranges (i.e., for circumstances such as administration of a loading dose). In terms of oral dosing, a clear formulation effect was observed among the examined dosage forms. Commercially available tablets produced erratic absorption and poor bioavailability. Maximum drug levels were subtherapeutic (the therapeutic range is 4–12 μg/mL) and were manifested only hours after drug administration. The low CBZ AUC, CBZ:CBZE AUC ratio, and similar or elevated CBZE AUC obtained after oral tablet treatment compared with iv CBZ·HPβCD are consistent with extensive metabolism as well as limited gut availability of CBZ due to its poor aqueous solubility in the tablet matrix. In addition, CL_R data suggest prolonged absorption of CBZ, which extended through the terminal elimination phase giving flip-flop behavior. Significantly better performance was provided by both liquid oral dosage forms, which were associated with more consistent, more rapid, and a greater extent of CBZ absorption. Both the solution and suspension approximately doubled the oral bioavailability compared with the tablets, generating an absolute bioavailability of 50%.

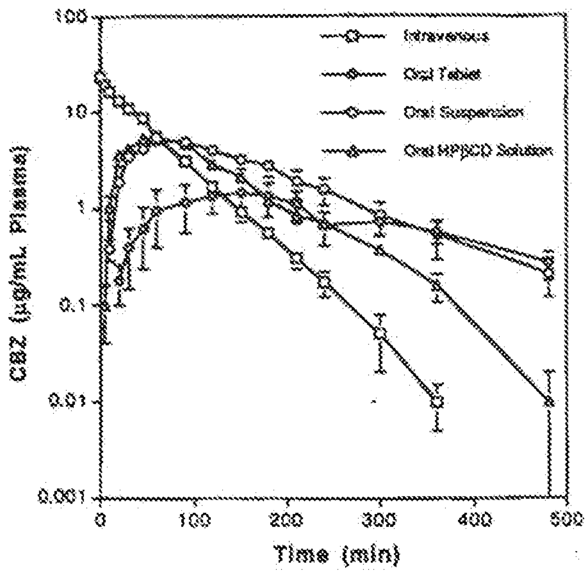

Figure 3—Disposition of CBZ administered at a dose of 20 mg/kg after either iv administration [in 22.5 (w/w) HPβCD] or oral treatment with tablets, a suspension, or an oral solution of CBZ in HPβCD. Plasma levels of CBZ were below the limit of detection by 24 h for all treatments. Data are expressed as mean ± SEM (n = 5).

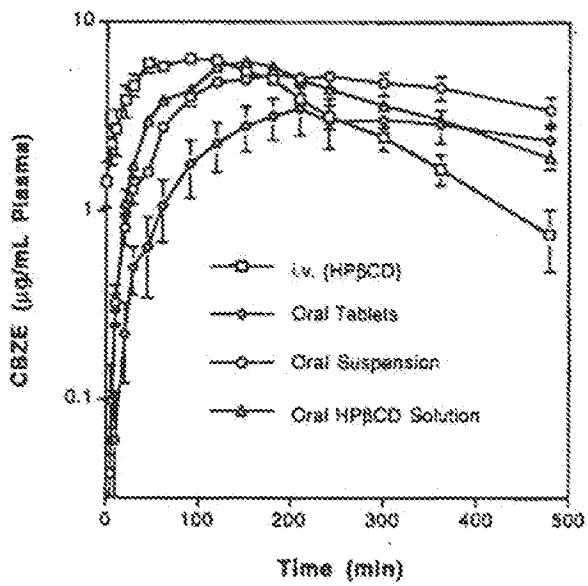

Figure 4—Disposition of CBZE after administration of CBZ at a dose of 20 mg/kg either by iv treatment [in 22.5% (w/w) HPβCD] or by oral dosing with tablets, a suspension, or an oral solution of CBZ in HPβCD. Plasma CBZE levels were 0.25 ± 0.09 (oral tablet), 0.14 ± 0.04 (suspension), and 0.52 ± 0.16 (HPβCD solution) at 24 h. Data are expressed as mean ± SEM (n = 5).

These data suggest that a cyclodextrin-based formulation of CBZ may provide an acceptable parenteral formulations as well as oral dosage form that are at least as bioavailable as suspensions. With the growing acceptance of cyclodextrins as excipients and the presence of HPβCD in formulations rapidly approaching regulatory approval, these methodologies may be beneficial in generating dosage forms not only for CBZ but also for a variety of other drugs that are difficult to formulate.[16,28,29]

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Preparation, Characterization, and Anesthetic Properties of 2-Hydroxypropyl-β-cyclodextrin Complexes of Pregnanolone and Pregnenolone in Rat and Mouse

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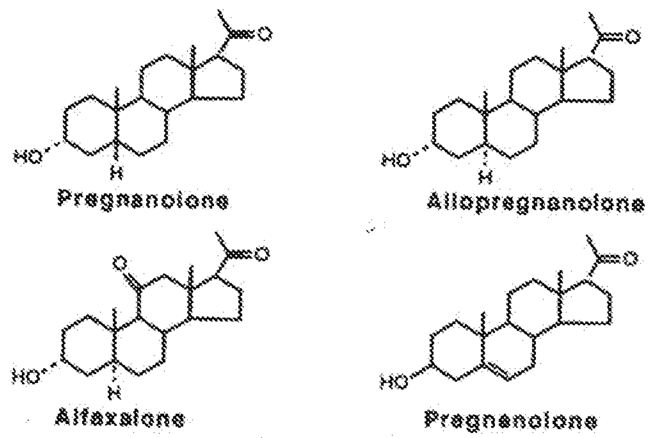
Abstract ☐ Prototype formulations of the progesterone derivatives pregnanolone and pregnenolone were prepared by solubilizing the steroids in 2-hydroxypropyl-β-cyclodextrin (HPβCD). The aqueous solubility of the steroids was increased as a function of HPβCD concentration generating linear (A_L) or curvilinear (A_P) phase–solubility profiles. While the solubility of pregnanolone could not be increased with the addition of water-soluble pharmaceutical polymers, the concentration of pregnanolone in HPβCD was increased more than 60% by the addition of small amounts (0.10%) of (hydroxypropyl)methylcellulose. Mice studies found that while pregnanolone was highly potent in an HPβCD vehicle, pregnenolone was devoid of activity. Since pregnenolone and pregnanolone differ marginally in structure and physicochemical profile, the data suggest that these derivatives interact via a specific receptor and not via nonspecific membrane perturbations. Sex differences in the action of the pregnanolone complex was observed in that parenteral (iv and ip) drug administration was more effective in males than females. These data are in contrast to observations made in the case of alfaxalone, a related steroid anesthetic, in which the sex difference favored female animals. On the other hand, females appeared to be more sensitive to the effects of the pregnanolone complex when administered orally. Finally, parenteral pregnanolone was more toxic to males than females with LD_{50} (iv) values of 355 and 548 μmol/kg, respectively.



Structures 1–4

Introduction

Various synthetic and naturally occurring progesterone derivatives exert significant pharmacological activity including anesthetic, anxiolytic, hypnotic, and anticonvulsant action, even though these compounds are inactive as glucocorticoids, mineralocorticoids, or sex steroids.[1,2] The potential usefulness of these derivatives has led to their clinical exploitation. Among the most potent of these "neurosteroids" are the naturally occurring isomeric progesterone A-ring reduction products 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and 3α-hydroxy-5β-pregnan-20-one (pregnanolone) as well as the synthetic derivative 3α-hydroxy-5α-pregnane-11,20-dione (alfaxalone).[3–10] These steroids are associated with rapid modulation of neuronal excitability, most likely via potentiation of GABA interaction with the $GABA_A$–benzodiazepine receptor complex.[11–13] These actions, which in some instances are similar to those of a barbiturate, include an increased open time for the associated chloride ion channel resulting in membrane hyperpolarization.[14]

The development of these products has been significantly impaired by their physicochemical properties. In particular, the steroids are very poorly water-soluble, complicating the configuration of safe and useful parenteral dosage forms. Initially, attempts to develop these steroids for anesthesia took the form of water-soluble prodrugs. The 21-hemisuccinate of 5β-pregnane-3,20-dione (hydroxydione) as well as the 3-phosphate salt of 3α-hydroxy-5β-pregnane-3,20-dione were both examined clinically.[15–18] Unfortunately, hydroxydione was removed from the market due to a series of side effects including thrombophlebitis and pain upon injection. The phosphate prodrug was eliminated as a drug candidate because of paraesthesia related to the neck and arms. In addition, both prodrugs were slow in eliciting anesthesia and hydroxydione was associated with a prolonged duration of action.[19,20] Other attempts to generate water-soluble anesthetic steroids have taken the form of amino-containing analogs such as 11α-(*N*,*N*-dimethylamino)-3β-ethoxy-3α-hydroxy-5α-pregnan-20-one (minaxolone) which is supplied as its citrate salt.[21] This steroid is reported to produce reasonable anesthesia but with a recovery profile inferior to that of alfaxalone. Minaxalone was halted in its development due to potential carcinogenicity.[19,20]

Of the poorly water-soluble derivatives, alfaxalone was highly regarded for various beneficial properties including rapid induction and offset of anesthesia and a good maintenance profile.[9,22,23] The limited aqueous solubility of alfaxalone required that it be formulated in Cremophor-EL, a nonionic detergent.[9] The use of this excipient is problematic due to destabilizing effects on mast cells with subsequent histamine release.[24–26] The anaphylactoid reaction produced by the formulation was severe enough that the product intended for human use, Althesin, was removed from the European market in the mid-1980s. There was reasonable evidence that it was the excipient, not the solubilized steroid, that was responsible for the adverse effects.

The most potent of the progesterone-derived neurosteroids are pregnanolone and allopregnanolone. Since early animal studies suggested that pregnanolone was the most potent anesthetic pregnane, this compound was first selected for evaluation.[27,28] While development of this product has been slow, a recently described emulsion formulation has furthered the clinical evaluation of the agent.[19,29–31] Unfortunately,

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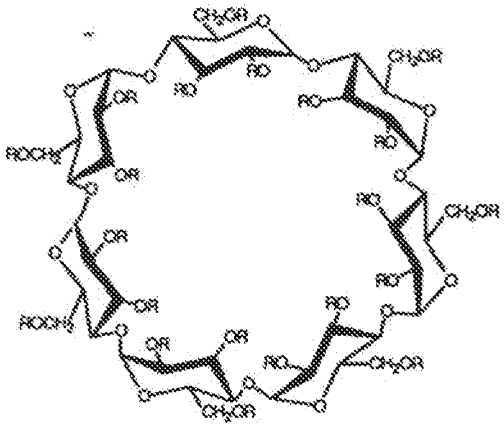
lipid-based emulsions suffer from several limitations including poor physical stability, the potential for embolism, and pain on injection.[32] One solubilization approach which may obviate the need for either detergents or emulsions is the use of water-soluble cyclodextrins. Cyclodextrins are cyclic oligomaltoses that provide for drug solubilization through the formation of dynamic inclusion complexes.[33] While β-cyclodextrin, the glucose heptamer, is poorly water-soluble, various chemical derivatives are highly water-soluble and useful. Thus, 2-hydroxypropyl-β-cyclodextrin (HPβCD) has been widely examined for its solubilizing and other beneficial properties.[34-37]



$R = O(CH_2CH(CH_3)O)_nH$

$n = 0,1,2...$

2-Hydroxypropyl-β-cyclodextrin

Structure 5

The starch derivative has been shown to be safe in various subacute and subchonic evaluations when administered orally or intravenously, allowing its use in both preliminary and advanced human clinical trials.[37-39]

Several studies have also suggested that HPβCD may be useful as an excipient for neurosteroids. Alfaxalone has been successfully formulated using a cyclodextrin-based dosage form which was shown to be safe in a Cremophor-sensitive species such as the dog.[40,41] Given the potential usefulness of aqueous HPβCD formulations of anesthetic steroids, a study was designed to examine the feasibility of complex formation with pregnanolone and pregnenolone (3β-hydroxypregn-5-en-20-one), the 5,6-unsaturated derivative. Studies were configured to evaluate the effect of HPβCD complexation on the aqueous solubility of the steroids as well as to examine the effects of added hydrophilic polymers on the efficiency of solubilization. Subsequently, the formed complexes were characterized as to their anesthetic potential and toxicity in rat and mouse models. Finally, the practicality of various HPβCD-containing pregnanolone formulations for application to human use was discussed.

Experimental Section

Materials—Pregnanolone and pregnenolone were obtained from Sigma Chemical Co. (St. Louis, MO). 2-Hydroxypropyl-β-cyclodextrin (HPβCD) was obtained from Roquette (Lestrem, France) and was characterized as having a degree of substitution of 4.2. Other materials used in these study included (hydroxypropyl)methylcellulose 4000 (HPMC) and poly(vinylpyrrolidone) 40 000 (PVP) (Mecobenzon, Denmark), (carboxymethyl)cellulose sodium salt (CMC) (Norsk Medicinaldepot, Norway), and dextran 40 000 (obtained by dilution of Onkovertin, B. Braun Melsungen AG, Germany). log P values were calculated according to the method of Bodor et al., which utilized an 18 parameter nonlinear model (Table 1).[42-44]

Solubility Studies—Two types of solubility trials were conducted. In the first, the solubility of pregnanolone and pregnenolone was determined using various aqueous solutions of HPβCD (5, 10, 20, 25, 30, 40, and 50% w/v). In these studies, the cyclodextrin solutions were prepared using 18.6 Ω deionized water (Barnsted Nanopure II Ultrapure Water systems). An excess of the steroid of interest was added to 10 mL of the appropriate cyclodextrin solution and mixed on a shaking table (Eberbach Corp.) for 3–5 days at room temperature. The resulting suspensions were then centrifuged and filtered through 0.45 μm polyvinylidene difluoride membranes (Nihon Millipore Kogyo, Yonezawa, Japan) and analyzed using UV spectrophotometry. The data represent the mean of two replicate samples. In a second set of experiments, the effect of various polymeric additives was assessed. In these trials, an excess of the steroid of interest was added to solutions of HPβCD (0, 1, 2.5, 5, 7.5, and 10% w/v) in the presence or absence of various pharmaceutical polymers including HPMC (0.1%), PVP (0.1 and 0.25%), CMC (0.25%), and dextran (0.25%). The suspensions were sonicated in an ultrasonic bath for 1 h and then autoclaved in sealed glass ampules at 120 °C for 20 min. The samples were then equilibrated at room temperature for at least 3 days. The suspensions were then filtered through an 0.45 μm polyvinylidene difluoride membrane, diluted with aqueous methanol (70% v/v), and analyzed by HPLC. In most cases, the data represent the mean ± standard deviation of three replicates.

Aqueous solubilities (S_o) and stability constants (K_c) were estimated from the solubility data ([M] of HPβCD versus [M] of steroid solubilized) using phase–solubility analysis. The initial linear portion of the phase–solubility curve was fitted to a straight line. The y-intercept gave the S_o, and the K_c was obtained by the following equation:

$$K_c = \frac{\text{slope}}{S_o(1 - \text{slope})}$$

Values for the S_o determined in this way were in good agreement with solubility values determined directly from aqueous media (Tables 2 and 3).

In preparing the freeze-dried complexes, an excess of pregnanolone or pregnenolone was equilibrated in a 50% w/v solution of HPβCD for 5 days, at which time the suspension was filtered. The filtrates were frozen in liquid nitrogen and lyophilized (Labconco model 18 freeze-drier). The solid complex was then milled by passage through a 60 mesh (particle size ≤ 250 μm) sieve. The degree of drug incorporation was then determined by HPLC to be 101.3 mg/g in the case of pregnanolone and 56.0 mg/g in the case of pregnenolone.

Analytical Methodology—Pregnanolone and pregnenolone were quantitated using two methods. For the initial phase solubility profile, UV analysis was performed. Pregnanolone and pregnenolone were analysed using an HP 8451A diode array spectrophotometer. Standard curves were prepared using methanol at a wavelength of 290 nm and were linear (r > 0.999) over the range of concentrations of interest. In several experiments, the HPβCD background absorbencies were determined by addition of appropriate volumes of solutions of HPβCD at appropriate concentrations to the methanol matrix. For analysis of the solubilized steroids, small volumes (1–50 μL) of the steroid-containing solutions were added to 2.75 mL of methanol contained in a screw-top cuvette. The absorbance of the solution at 290 nm was recorded and the concentration determined using a standard Beer's law plot. The concentration was then converted to the amount of the steroid added to the cuvette which was then used to determine the concentration of the steroid in the original HPβCD-based solution. Analyses of pregnanolone and pregnenolone in other systems were accomplished using HPLC. The system configuration consisted of a Milton Ray ConstaMetric 3200 solvent pump, a Rheodyne 7125 injector, and a Spectro Monitor 3200 UV/vis variable wavelength detector (209 nm for pregnanolone and

Table 1—Calculated Molecular Dimensions and Properties for Pregnanolone and Pregnenolone

Property	Pregnanolone	Pregnenolone
log P	3.44	3.66
Molecular volume (Å^3)	328.5	322.7
Surface area (Å^2)	373.8	369.9
Ovality	1.63	1.63
HOMO energy (eV)	−10.16	−9.35
LUMO energy (eV)	0.94	0.90
Heats of formation (kcal/mol)	−149.07	−124.66

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Table 2—Effect of Various Water-Soluble Polymers on Solubilization of Pregnanolone by HPβCD

	Solubility (mg/mL ± SD)						
		HMPC		PVP			
HPβCD (% w/v)	No Additive (H_2O)	0.10%	0.25%	0.10%	0.25%	CMC: 0.25%	Dextran: 0.25%
0.0	0.008		0.007	0.008		0.812 ± 0.164	
1.0	0.188	0.161	0.107	0.220	0.183	1.052	0.728 ± 0.065
2.5	1.582	0.688		1.507 ± 0.349		1.943 ± 0.238	1.004
5.0	2.454	1.545	1.164	2.679	3.270 ± 0.199	3.249 ± 0.353	1.691 ± 0.258
7.5	5.654 ± 0.378	3.012 ± 0.334	2.377 ± 0.249		5.201 ± 0.478	4.665 ± 0.280	2.992 ± 0.193
10.0	7.399 ± 0.267	8.582 ± 0.992		5.915	6.656 ± 0.445	5.962 ± 0.425	4.666 ± 0.322

Table 3—Effect of Various Water-Soluble Polymers on Solubilization of Pregnenolone by HPβCD

	Solubility (mg/mL ± SD)						
		HMPC		PVP			
HPβCD (% w/v)	No Additive(H_2O)	0.10%	0.25%	0.10%	0.25%	CMC: 0.25%	Dextran: 0.25%
0.0	0.041 ± 0.015	0.043 ± 0.008	0.046 ± 0.009	0.042	0.048	0.048 ± 0.0.009	0.047 ± 0.008
1.0	0.071 ± 0.002	0.049 ± 0.010	0.055 ± 0.009	0.106 ± 0.007	0.077 ± 0.014	0.127 ± 0.013	0.207 ± 0.010
2.5	0.810 ± 0.038	1.000 ± 0.054	0.809 ± 0.086	0.816 ± 0.048	0.700 ± 0.187	0.836 ± 0.066	0.528 ± 0.029
5.0	1.745 ± 0.033	2.314 ± 0.072	1.932	1.604 ± 0.062	1.569 ± 0.085	1.751 ± 0.043	1.253 ± 0.045
7.5	2.236 ± 0.071	3.614 ± 0.122	3.349 ± 0.278	1.900 ± 0.066	2.493 ± 0.117	1.649 ± 0.102	1.982 ± 0.056
10.0	2.949 ± 0.055	4.732 ± 0.194	4.623 ± 0.086	3.195 ± 0.070	3.285 ± 0.068	2.982 ± 0.119	2.666 ± 0.103

210 nm for pregnenolone). Compounds were eluted on a Beckman ODS 5 μm particle size, 15 cm × 4.6 mm i.d. analytical column. The flow rate was 1.5 mL/min, and all determinations were performed at ambient temperature (20–25 °C). Pregnanolone was eluted using a mobile phase containing acetonitrile:tetrahydrofuran:water (80:1:19) while pregnenolone was eluted using a mobile phase containing acetonitrile:tetrahydrofuran:water (65:1:34). The retention times for pregnanolone and pregnenolone in the above systems were 4.0 and 2.9 min, respectively. Standard curves of pregnanolone and pregnenolone were linear over the concentration range of interest. UV and HPLC methods were highly consistent (e.g. in two separate experiments, the concentration of pregnenolone (at 10% HPβCD) was 3.01 mg/mL (UV) and 2.95 mg/mL (HPLC) while for pregnanolone (at 10% HPβCD) the measured concentration was 7.46 mg/mL (UV) and 7.40 (HPLC) mg/mL.

Pharmacological Studies—Studies were conducted using either male ICR (Charles River-derived; CD-1) mice weighing 30–35 g or in male and female Sprague-Dawley rats weighing 175–200 g (both procured from Harlan Sprague-Dawley, Inc., Indianapolis, IN). Animals were housed in our vivarium and were maintained at 23 ± 2 °C at a relative humidity of 55–70%. The light:dark cycle was 12 h:12 h with lights on at 0700 h. Animals were provided rodent chow (Teklad 4%) and tap water ad libitum.

Male mice (n = 6) were used for screening and received various doses of the anesthetic steroids (0, 12.5, 25, 75, and 100 μmol/kg or approximately 0, 4, 8, 24, and 32 mg/kg) in HPβCD via the lateral tail vein. Animals were restrained in a "Broome"-type holder for drug administration. Drugs were dissolved in an aqueous solution (22.5% w/v) of HPβCD yielding stock solutions of 6.3 and 11.4 mg/mL for pregnenolone and pregnanolone, respectively. The dose was adjusted by dilution with aqueous cyclodextrin (22.5% w/v) and the volume was maintained at 3.0 mL/kg (providing an HPβCD dose of 775 μg/g). Parameters that were examined included loss of righting response, length of sedation, and behavioral changes associated with anesthesia.

Male and female rats (n = 6–8) received pregnanolone (in doses ranging from 36 to 1438 μmol/kg) via various administration routes including intravenous, intraperitoneal, and oral (iv, ip, and po, respectively). For *iv* administration, animals were restrained in a "Broome"-type holder. Pregnanolone was provided in a 43.5% w/v solution of HPβCD which was adjusted for a particular dose with a diluent solution of the cyclodextrin (also 43.5% w/v). The volume of the formulation was maintained at 1.0 mL/kg providing an HPβCD dose of 435 mg/kg. Intraperitoneal injections were administered by conventional means with a 25 gauge needle and utilized HPβCD solutions of pregnanolone administered at a volume of 8.0 mL/kg or an excipient dose of 3.48 g/kg. Rats receiving oral pregnanolone were transiently intubated with a 18 gauge, 2 in. long, 2.25 mm ball stainless steel, bent-tube type feeding needle (Popper and Sons, Inc.). The needle was positioned through the esophagus and past the cardiac sphincter of the stomach. The pregnanolone solution was contained in HPβCD (43.5% w/v) and was delivered at 14.8 mL/kg or 6.44 g of HPβCD/kg. Parameters that were recorded included loss and reestablishment of righting responses, time of anesthetic induction, and total sleeping time.[8,20] Induction (sleep onset) was defined as the time from drug administration to loss of righting reflex and the sleep time was the time from the loss of the righting reflex until the animals were plantigrade on all four legs. The significance of differences in mean induction and sleeping times was analyzed using analysis of variance (ANOVA) with post-hoc Tukey's comparison. For all tests, the level of probability was $p < 0.05$.

Toxicity was examined in an acute lethality study. Drug was administered either iv, ip, or po, and animals were observed for 24 h for lethality. The percent survival (out of six to eight animals) was determined and plotted as probits as a function of log dose. The median lethal dose (LD_{50}) was then determined according to the method of Miller and Tainter.[45]

Results and Discussion

Two potential drug candidates derived biochemically from progesterone, i.e. pregnanolone and pregnenolone, were examined in this study. These derivatives are relatively lipophilic with calculated log P values of 3.44 and 3.66, respectively (Table 1). Such high lipophilicity limits the water solubility of the two steroids which was determined to be 0.008 mg/mL for pregnanolone and 0.041 mg/mL for pregnenolone. The aqueous solubility of both pregnanolone and pregnenolone (Figure 1) was dramatically affected by the presence of varying concentrations of HPβCD. The phase–solubility profile generated was not significantly different from linearity (A_L) in the case of pregnanolone and exhibited a positive deviation from linearity (A_P) in the case of pregnenolone. In the latter case, the solubility of the steroids could be best fitted to a quadratic model.[46] In any case, a 50% HPβCD provided for an 18.4 mg/mL solution of pregnenolone (or a 550-fold increased in solubility compared to a simple aqueous system) and a 38.8 mg/mL solution of pregnanolone (or a 4850-fold increase in solubility). Using the initial (linear) portion of the phase–solubility profiles, the equilibrium constants for the formation of the 1:1 complex ($K_{1:1}$ of K_c) was estimated to be